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SUBACUTE ENCEPHALITIS (DAWSON)

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Since Dawson, of the U.S.A., in 1933 and 1934 described the first cases of subacute inclusion encephalitis several other investigators have reported cases of the disease.

Pette & Döring reported some cases of panencephalomyelitis in 1939, and van Bogaert described subacute sclerosing leukoencephalitis in 1945. Most authors now concur that cases of Dawson's inclusion encephalitis cannot be distinguished clinically from van Bogaert's leukoencephalitis, and that panencephalomyelitis may belong to the same group.

Cases have been reported from the U.S.A., Canada (van Buren 1954), England (Greenfield 1950), Belgium, Germany and Bulgaria (Ousounov et al. 1957). For a more detailed bibliography, the reader is referred to Landau & Luse (1958).

The disease is characterised by its subacute course, the clinical picture and typical changes in the EEG. Intranuclear inclusions were found in only about one half of the reported cases. The disease usually occurs during the first two decades of life.

The present investigation does not add to our knowledge of the clinical picture. However, in four of our five cases, cortex biopsy was performed, which gave a possibility of making the diagnosis during life, and the diagnosis was secured in this way in two of these four cases.

CASE REPORTS

The five cases considered here were so uniform that the clinical history and laboratory data can be illustrated by a detailed description of just one case. However, the results of the histological examination must be described in all five cases, since we wish to discuss in detail the significance of the intranuclear inclusions.

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Case No. 1.

A girl, aged 14 years, was admitted to the Department of Neurology on Aug. 1, 1956. Her past history was essentially that of good health, apart from the fact that, at the age of about seven years, she had been admitted to hospital with symptoms which were supposed to be due to nervousness.

For about four months prior to admission she seemed somewhat changed; she was more preoccupied and indolent, and often dropped objects she held in her hands. On a few occasions brief faintings and vomiting had occurred. At first, the condition was thought to be "nervousness", also because the symptoms developed shortly after she had taken up a more strenuous job. She was referred for a psychiatric examination, at which a hysterical psychosis was suspected, but shortly afterwards EEG showed an abnormal paroxysmal pattern suggestive of epilepsy.

On admission, she gave the impression of mental tension, and her behaviour was rather awkward; thus, she might undress in queer places, pass urine on the floor, etc. Apart from blurring of the optic discs, no neurological abnormalities were revealed. Gradually, the condition became more and more marked by absence-like attacks accompanied by generalised myoclonic jerks, at first often occurring in close succession, but later with a striking periodicity. For months she had thus short transient episodes of unconsciousness seven to ten times per minute with simultaneous jerking movements. Mental and motor deterioration developed; it became increasingly difficult to make contact with her, and at last her existence appeared to be on a purely vegetative level. Severe quadriparesis developed, accompanied by a generalised increase in the muscle tone, left-sided foot clonus, Babinski response, bilateral papilloedema of 1 dioptre and, later, moderate opistotonus.

Laboratory findings. — A week after admission the spinal fluid showed 16/3 mononuclear cells and total protein 60 mg/100 ml (Lowry); a collargol test revealed a paretic curve. White blood cells 8040 per c.mm with a normal distribution. The blood pressure and sedimentation rate were normal.

Electro-encephalography was performed on 14 occasions during the period from Aug. 3 to Nov. 16, 1956, and once before admission. The findings are illustrated by the examples in Figure 1. The curve was constantly abnormal and characterised by paroxysms; at first there was some alpha activity, which decreased

Fig. 1.

EEG (monopolar technique) from Case No. 1. The five upper records show the changes in the EEG pattern during the course. The bottom record is an electromyogram simultaneously with the paroxysms observed in the EEG.

terminally, so that the intervals between the paroxysms approached iso-electrical activity. The curves showed some fluctuation from examination to examination. At EEG with concurrent electromyography, a brief electromyogram was observed simutaneously with the paroxysms. The curve was not changed by sleep provoked by 600 mg of Tio-pentemal or by intravenous injection of 800 mg Tridione, whereas the intervals between the paroxysms were temporarily lengthened after intravenous injection of 100 mg of Lidocaine.

Ventriculography (Oct. 17). — The brain tissue was tougher than normal, and great resistance was particularly encountered periventricularly. The form and size of the ventriculograms were normal. During the examination a biopsy specimen was secured.

Biopsy. — The staining methods used in this and the following cases were: haematoxylin-eosin, v. Giesson-Hansen, gallocyanin chromalum, Weil, toluidine blue and azur-eosin. The colour of the inclusions given in the text always refers to the haemotoxylin-eosin staining.

The structure of the cortex was well preserved, but perivascularly it was markedly infiltrated with lymphocytes and macrophages. The ganglion cells showed all degrees of degeneration ranging from swelling to atrophy, and from chromatolysis to pyknosis. Most ganglion cells seemed to be irreversibly changed, and pronounced gliosis was present both in the grey and the white substances. The changes appeared to be equally advanced in the cortex and the subcortical layers. Distinct inflammatory reactions, especially in the perivascular areas, and oedema were seen in the meninges. Histological diagnosis: subacute meningo-encephalitis.

Scattered throughout the sections, the glia cells revealed a few lavender blue to bluish red intranuclear inclusions. The inclusions completely filled the nucleus, forcing the chromatin and nucleolus towards the periphery; no halo phenomenon was seen.

The patient became deeply comatose, and death occurred on Dec. 9, 1956.

Post-mortem studies. — The brain weighed 1525 g. The leptomeninges were slightly blurred. The brain was diffusely oedematous, but haemorrhages were absent in the leptomeninges. On frontal section, the cerebrum showed no grossly abnormal features, except in the occipital lobes, in which the white substance was somewhat spongy, with degeneration laterally and above the posterior horns. In the remainder of the cerebrum the pattern seen on the cut surface was perfectly normal. Nor were any grossly abnormal features observed in the pons, medulla oblongata or cerebellum.

Histological examination. — In some areas of the occipital lobes, both the cortex and the white substance showed pronounced changes, so that only a narrow rim of subpial proliferating glial tissue with large swollen astrocytes was left behind. The remainder of the cortex and white substance showed vessels with a variable degree of perivascular lymphocytic infiltration. The normal tissue was completely destroyed, and the tissue now consisted almost exclusively of monstrous protoplasmic astrocytes, a few fibrillary astrocytes, degenerated oligodendroglial cells and some lipoid-containing macrophages. In other areas, the cortex was fairly well preserved, while the changes were situated subcortically. The white substance showed complete or partial degeneration of the myelin sheaths. The process showed various stages of development, with gradual transition to brain tissue of a near-normal structure. Histological diagnosis: panencephalitis (Dr. Erna Christensen).

In spite of meticulous examination of sections from the superior frontal gyrus, hypothalamus and the temporal, inferior parietal and right occipital lobes, no inclusions were observed.

Serological Studies.

As already mentioned, the clinical features observed in all five cases were almost identical (Table 1), and so were the laboratory data. It should, however, be mentioned that in Cases No. 4 and 5 serological tests for leptospirosis were performed, in both with negative results. In Case No. 5, complement fixation tests for mumps and Q fever were also negative, and so was the Paul-Bunnell test. In Cases No. 1 and 2, the complement fixation test for ornithosis was negative. The cold agglutinin test was normal in Cases No. 2 and 4.

Finally, in Cases No. 2 and 4 a haemagglutination test for auto-antibodies was performed with erythrocytes coated with an antigen extracted from normal brain tissue, likewise with negative results. No attempts were made to isolate viruses.

In the remaining four cases, a brief description of the histological observations will suffice.

Case No. 2. — A biopsy specimen of the cortex of the occipital lobe was secured on Nov. 7, 1956. The pial cortex and white substance revealed pronounced perivascular infiltration with lymphocytes and some plasma cells and macrophages containing lipoid and blood pigment. The grey substance showed some degeneration of the ganglion cells and glial proliferation around the perivascular foci. In the white substance, diffuse swelling of the astrocytes was observed, whereas the oligodendrocytes showed some tendency to atrophy. The glial changes were most pronounced in the vicinity of the perivascular foci. Oedema and degeneration of the myelin sheaths were also found perivascularly. Histological diagnosis: severe meningo-encephalitis.

In the cortex and white substance, a few intranuclear inclusions were visible, usually filling the entire nucleus and of a bluish red colour.

Case No. 3. — Postmortem histological examination showed oedema of the leptomeninges and infiltration with lymphocytes and plasma cells. Most of the ganglion cells revealed a variable degree of degeneration. Proliferation of the astrocytes and variable perivascular infiltration with lymphocytes, macrophages and plasma cells were seen. There were no perivascular haemorrhages, but oedema with degeneration of the myelin sheaths was present. The aforementioned changes were most pronounced in the frontal lobe. Both macrophages and astrocytes contained lipoid, which was also found in the extracellular spaces.

Sections from the motor zone, temporal lobe, frontalt lobe and occipital lobe showed intranuclear inclusions in almost any stage of development ranging

Survey of the five ca

Case	Sex	Age (years)	Duration of symptoms (months)	Date of admission	Case history			
					Initial symptoms	Subsequent course	Date	Pressure (mm H_2O)
1	F	14	4	Aug. 1, 1956	Indolent, poor concentration, nausea, fainting	Myoclonic jerks, choked discs, quadriparesis, rigidity	Aug. 8, 1956	220
2	M	12	6	Aug. 6, 1956	Incoherent speech, poor concentration, mental disturbances, fainting	Myoclonic jerks, quadriparalysis, rigidity	Aug. 16, 1956	140
3	M	7	1	Oct. 30, 1952	Incoherent speech, poor concentration indolent, hallucinations, episodes of pallor	Aphasia, hemiplegia, myoclonic jerks, quadriparalysis, rigidity, terminal hyperthermia	Oct. 24, 1952 (v.-graphy)	
4	M	14	8	June 18, 1957	Fever?, fatigue, incoherent speech, impaired concentration, aggressive, irascible	Hemiparalysis, elation, perseveration, quadriparalysis, rigidity	June 25, 1957	165
5	M	12	6	Aug. 3, 1954	Dizziness, fatigue, excessive sleep requirements, faintings, transient blurring of vision	Choked discs, myoclonic jerks, quadriparalysis, rigidity	Sep. 24, 1954	160

from lavender blue and bluish red inclusions occupying the entire nucleus to bright red inclusions surrounded by a clear halo.

Case No. 4. — A biopsy specimen of the cortex was secured on June 28, 1957. Both in the pia, the cortical grey substance and the white substance, marked lymphocytic perivascular infiltration was seen; microglial cells, some of which contained lipoid, were also present. Both the cortical grey matter and the white substance revealed marked proliferation of astrocytes, in some places arranged in nests. The ganglion cells showed some degeneration, and satellitosis was present. The white substance exhibited slight degeneration of the myelin sheaths. Histological diagnosis: subacute meningo-encephalitis (panencephalitis).

Although a meticulous search was carried out, no inclusions were found.

Case No. 5. — A biopsy specimen of the cortex of the parietal lobe was secured on Jan. 26, 1955. It showed moderate fibrosis of the leptomeninges, but only a few lymphocytes were present. Atrophy and degeneration of the ganglion cells were present in the cortex; these changes were most pronounced in the deep layers, where increased proliferation of the glia cells was seen. In the subcortical layers and in the white substance, the myelin sheaths were almost completely degenerated, with pronounced proliferation of the gemistocytic astrocytes. Perivascular infiltrations of lymphocytes and microglial cells were observed, and infiltrations were also present in the vessel walls. The cells contained large amounts of lipoid, whereas no inclusions were demonstrated.

Studies of autopsy material (February 6, 1956) revealed a similar picture. The primary changes were supposed to be the perivascular inflammatory infiltration, which was followed by degeneration of the white substance and subsequent proliferation of the astrocytes. Histological diagnosis: panencephalitis.

Throughout the brain (sections from the frontal lobe, corpus striatum, calcarine fissure and hypothalamus) there were areas with intranuclear inclusions in both glial and nerve cells. The inclusions usually filled the nucleus completely, and the colour varied from bluish red to red.

As appears from Table 1, cortical biopsy was performed in relation to ventriculography in four of the five cases. In all four cases the biopsy specimen was taken from either the occipital lobe or the junction between the occipital and parietal lobes. This procedure confirmed the diagnosis in Cases No. 1 and 2.

A meticulous search did not reveal inclusions in autopsy material from Case No. 1, although this examination was performed only eight weeks after the biopsy. Conversely, the biopsy was negative for inclusion bodies in Case No. 5, while autopsy material revealed numerous areas with these bodies. However, it should be noted that these areas were mainly found in the deeper layers, and that it was not possible to find similar

...le encephalitis (Dawson).

...al fluid			Ventriculography		Biopsy	Electro-encephalography		Death	Autopsy
Pressure (mm H_2O)	Cells	Protein (mg/100 ml)	Paretic curve (coll.)	Pressure (mm H_2O)	Ventriculogram		Periodic paroxysms	Electromyogram simultaneously with paroxysms		
220	16/3	60	+	0	Normal	Oct. 17, 1956, lavender blue inclusions	+	+	Dec. 9, 1956	No inclusions
140	0/3	68	+	40—50	Moderate diffuse dilatation	Nov. 7, 1956, blue to bluish red inclusions	+	+	March 29, 1957	No autopsy
	132/3 and ...merous ...rythro-cytes			150—190	Dilatation suspected	No biopsy	+	?	March 16, 1953	Numerous blue and red inclusions
165	1/3	26	+	80—110	Normal	June 28, 1957, no inclusions	+	+	Aug. 15, 1958	No autopsy
160	40/3	70	+	450—550	Normal	Jan. 26, 1955, no inclusions	+	+	Feb. 6, 1956	Numerous bluish red to red inclusions

areas with inclusion bodies in autopsy material from the cortex of the occipital lobe. The interval between biopsy and autopsy was 13 months.

It should be mentioned that in Case No. 1 lavender blue inclusions occupied the entire nucleus with peripheral displacement of the nuclear chromatin, while Case No. 2 exhibited all transitional forms from lavender blue to purely eosinophilic inclusions of a characteristic type A appearance.

It was studied if it was possible to say anything as to the relationship of the lavender blue and eosinophilic inclusions to the phase of activity of the disease on the basis of objective clinical and laboratory findings. On the assumption that the development of inclusion bodies occurs as described by Crouse (see below), it would be resonable to expect that a patient in whom the biopsy specimen was secured in a more "acute phase" would reveal the early forms rather than the more mature varieties of inclusion bodies. However, such a correlation could not be demonstrated.

DISCUSSION

The clinical pictures observed in our patients were exactly like that described by Dawson in 1933. The disease usually occurs in the first two decades of life. It is of insidious onset, and the initial symptoms are often ascribed to nervousness. Mental disturbances, difficulties in school work, incoherent speech, fatigue, etc. develop, followed by various epileptic symptoms, such as lapse-like attacks with myoclonic jerks, which may recur periodically month after month, often with a frequency of five to ten per minute, and other forms of hyperkinesia. Speech disturbances or monotonous repetition of words or tunes gradually developing into dementia and severe paralysis of the limbs may be observed. The disease is fatal, death usually occurring within six to twelve months. However, cases in which the course proves to be chronic are known.

The cases considered by us tally exactly with this picture and do not reveal any new features. The laboratory findings with a slight increase in protein in the spinal fluid, a paretic collargol curve and no or only a slight increase in cells are also those usually observed. The EEG findings are likewise in conformity with the observations made since Radermecker (1949) and Cobb & Hill (1950) described the picture. The typical feature is an abnormal EEG pattern characterised by paroxysms, but with fluctuations from examination to examination, yet with a tendency to gradual transition to paroxysms at roughly regular intervals and low voltage between the paroxysms. A survey of the EEG findings is given,

for example, by Radermecker (1956), to whose paper the reader is referred.

INCLUSION BODIES

The meaning of the term inclusion body may vary; histologists often use it in a wide sense, so that any foreign material included within a cell is described as inclusion bodies. On the other hand, the virologists use the term for special intracellular formations which are specific for certain viruses. Inclusion bodies may be demonstrated in practically all cases of acute infection by the virus concerned, but are absent in uninvolved tissue.

According to their localisation in the cell, inclusion bodies may be classified as either intracytoplasmic or intranuclear. Intracytoplasmic inclusion bodies, which occur in smallpox, rabies, psittacosis and many other diseases, are left out of account here.

Intranuclear inclusion bodies may be subdivided into type A and type B (Cowdry 1934).

Type A is either hyaline or granular and gives rise to pronounced nuclear changes. The chromatin and nucleolus are displaced into the periphery of the nucleus, and the typical form displays a clear zone (halo) between the inclusion body and the chromatin.

Type B gives less violent changes. The inclusion bodies are smaller, and several inclusions are often present in the same nucleus. The displacement of the chromatin is less pronounced, even though a halo may occasionally be present around the inclusion body.

Intranuclear inclusion bodies of type A, as they appear also in the subacute inclusion encephalitis described by Dawson, may be due to various viruses, principally herpes virus, but also zoster and chickenpox viruses. In tissue culture, not only less well-known viruses (such as salivary-gland virus), but also adenovirus and measles virus give rise to the development of eosinophilic intranuclear inclusion bodies.

According to Crouse et al. (1950), the intranuclear inclusion bodies developing in infections due to herpes virus are first seen as two small Feulgen-positive areas, which then fuse, forming a Feulgen-positive basophilic homogeneous inclusion body, which fills up the entire nucleus and displaces the chromatin into the periphery. Later, the inclusion body shrinks and develops into the characteristic type A variety, which is eosinophilic, Feulgen-negative and with a distinct separation from the chromatin by a clear zone, the so-called halo.

The nature of the inclusion bodies is not as yet fully clarified. The occurrence of the inclusions has been explained either as a cellular reaction to the virus infection, *i. e.*, a cellular product, or as a direct accumulation of virus particles; in other words, the inclusion bodies should be intracellular colonies of virus particles. During recent years, evidence in favour of the latter concept has been revealed in studies by the electron microscope, in which accumulations of virus particles have repeatedly been seen to form crystal-like formations in the inclusion bodies. These observations have been made both in intracellular and intranuclear inclusion bodies (Tajim 1957, Morgan et al. 1956).

Fig. 2.
Nerve cells with intranuclear inclusions (arrow indicating the localisation of the nucleolus). Haematoxylin-eosin, × 900.

The demonstration of inclusion bodies may be utilised as a diagnostic aid in the direct study of clinical material, for example, in rabies, trachoma and inclusion conjunctivitis. On inoculation of clinical material into experimental animals, later demonstration of inclusion bodies may be used for diagnostic purposes, for example, in smallpox (Paul's test) and psittacosis. However, it may be queried if demonstration of inclusion bodies should invariably be interpreted as unequivocal evidence of the presence of a virus infection. In the slightly older literature, it is reported that inclusion bodies have been observed in tissue from apparently healthy individuals, especially children. In the light of our present knowledge of a number of previously unknown viruses (including salivary-gland virus) and of latent viral infections, the significance of the occurrence of inclusion bodies in apparently normal persons must be assessed with great caution.

In Dawson encephalitis, it may be extremely difficult to disclose the presence of inclusions.

In some cases, they are seen everywhere in the cerebrum, and the diagnosis is thus easy; but in most cases, they are sparse, although with a certain tendency to accumulation in small areas, and the search must often be continued for hours before they are found. In the present series, the sections were searched systematically three or four times before the histologist ventured to describe them as negative for inclusions. In one case (Fig. 2), an area with typical inclusion bodies was disclosed only after histological studies extending over more than 20 hours.

As already mentioned, the inclusions observed in Dawson encephalitis are intranuclear and eosinophilic, but a few investigators *(e.g.,* Malamud et al. 1950) have occasionally observed lavender blue inclusions occupying the entire nucleus, *i.e.,* similar to the first phase in the development described by Crouse.

As previously pointed out, this form was also observed in the present series, either alone or together with characteristic type A inclusions, for which reason we made a diagnosis of inclusion encephalitis in the cases which revealed only lavender blue inclusions.

In order to make a diagnosis of subacute inclusion encephalitis (Dawson), it must — if this designation is to be maintained — be required that inclusion bodies have been demonstrated. However, in practice, it appears that the diagnosis may be made with a great degree of certainly only on the basis of the clinical course, the EEG and other investigations. The actual finding of inclusions confirms the diagnosis, whereas failure to reveal inclusion does not exclude this disease. However, according to the observations made in the present series, the occurrence of inclusion bodies seems to be inconstant in the individual patient, as shown in our typical example, Case No. 1, in which inclusion bodies were unquestionably demonstrated in the biopsy specimen, whereas they could not be found in the autopsy material.

In the subacute sclerosing leukoencephalitis described by van Bogaert, no inclusions were found in the first cases, but a later reappraisal of the sections showed that such inclusions were present in some of the patients.

Thus, the demonstration of inclusion bodies may be difficult, and according to the results of the present study, their occurrence also seems to be inconstant. On the basis of our own experience and the reports published in the literature, we therefore find that it would be reasonable to discard the designation inclusion encephalitis and to adopt instead the term subacute encephalitis (Dawson), and that it is justified to make this diagnosis in the absence of demonstration of inclusion bodies. Incidentally, the designation "cytomegalic inclusion encephalitis" used by Campbell et al. (1952) should also be avoided, since "cytomegalic inclusion disease" is already used for a generalised salivary-gland virus infection.

The aetiology of Dawson encephalitis is unknown. In the cases in which attempts have been made to isolate the virus, the results have usually been negative. A few reports on isolation of herpes virus are available, but as this virus in ubiquitous, and as the isolation has been successful only on a few occasions, these reports must be assessed with great caution. Moreover, some of the isolation experiments have been performed on laboratory animals (especially mice), and as adenoviruses are not pathogenic for ordinary experimental animals, this group of viruses cannot possibly be demonstrated in such experiments.

It should be noted that the viruses which give rise to intranuclear inclusion bodies of type A are very prone to cause latent infections, which undoubtedly renders the demonstration of the viruses concerned very difficult.

SUMMARY

Five cases of subacute encephalitis (Dawson) are reported. In four of the patients, biopsy specimens were obtained, and in two of these it was possible to demonstrate intranuclear inclusion bodies. In another two cases, inclusion bodies were revealed in autopsy material, and only in one case was the diagnosis made on the basis of clinical observations.

The clinical pictures and laboratory findings were so uniform that a detailed description of only one case has been given, whereas the histological findings in biopsy and autopsy material from all five cases are reported.

The aetiology of the disease is unknown, but it is reasonable to assume that the causative agent is a virus. The significance of the demonstration of inclusion bodies in support of this view is discussed.

Moreover, it is suggested that the designation "subacute inclusion encephalitis" should be discarded, partly because the occurrence of inclusion bodies, as appears from this series, is inconstant, and partly because the diagnosis *may* be made only on the basis of the clinical course and laboratory findings, especially EEG.

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NEUROINFECTIONS IN ASIAN INFLUENZA

By BENT de FINE OLIVARIUS and MOGENS FOG

The first official reports on cases of socalled Asian influenza came May 3, 1957 from Hongkong, where 20 per cent of the population were suffering from the disease. The course of the infection seemed benign. On May 4, 1957 similar cases were reported from Singapore, and May 9, a virus was isolated there. The epidemic is presumed to have started in continental China although exact accounts are lacking. The disease showed tendency to rapid spreading and with almost explosive speed the epidemic followed the direction Japan — Australia — India and finally the U. S. and Europe, where the first cases were seen in Holland.

While the epidemic was thus widely spread and affected a great proportion of the population, in most parts of the world it continued to have a mild course. Considering the great number of affected persons the number of complications were hardly relatively larger than in former less extensive influenza epidemics.

In this country the medical statistic reports for the years 1956 and 1957 (5) show an increase in the number of influenza cases to five times that of 1956, and the number of fatal cases were ten times greater.

In a description of an epidemic in a Canadian girls' camp Rebham (15) reported that "no serious sequelae are known". Severe complications, especially from the respiratory tract, were later published, however. Thus, Giles & Shuttleworth (10), in a report on the postmortem findings in 46 influenza deaths, found an overwhelming predominance of pneumonia (79 per cent) as the cause of death in these patients. Hermann & al. (11) reported 23 consecutive cases of unexplained, unusual or unattended deaths reported to the Denver coroner's office in a three week period in October 1957 during the Asian influenza epidemic. An acute respiratory inflammation was considered to be the cause of death, and autopsy revealed an unusual degree of laryngo-tracheo-bronchial inflammation and a variety of pneumonias. The cases comprised all age groups and death usually supervened after a short period of illness, mainly, however, in persons who had not been submitted to medical care or in persons suffering from chronic diseases.

It has been shown that these fatal pneumonias are often due to superinfection with staphylococcus aureus. Several fatal cases of pneumonia with a fulminant course have been reported in this country. (Andersson & Lorenzen (2), Ryssing (16)).

Few reports on neuroinfections in Asian influenza are found in the literature. Lawrence & Jones (12) were presumably the first to report on this complication. They describe the case of a 40-year-old man who the day after evidently having recovered from an attack of Asian influenza became restless with clouded sensorium and unintelligible speech. Aside from mental clouding no neurological manifestations were present, but spinal fluid showed pleocytosis. The patient became mentally clear in the course of a few days. The authors furthermore drew attention to several similar cases, which had been observed by them, presenting sudden complete disorientation and confusion for some hours after the influenza symptoms have subsided.

Bennett & Turck (3) described the case of a 16-year-old boy, who during the course of an Asian influenza was mentally clouded and gradually became comatose at the same time as he presented signs of a severe pneumonia. The patient died on the seventh day and postmortem examination showed cerebral edema and hyperemia with perivascular hemorrhages. This case is listed as a case of encephalitis, but it is a question if it has not been a toxic encephalopathy secondary to the pneumonia.

Dubowitz (6) and Anderson & Jaros (1) have described two and three cases, respectively, of well documented encephalitis.

Gerstenbrand & al. (9) from the Psychiatric-neurological University Clinic in Vienna

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have collected a material of 17 cases with encephalitis complicating influenza. Nearly all patients had recovered from an evidently non-complicated influenza infection at the time when symptoms of encephalitis occurred. These authors distinguish two types of encephalitis essentially different in course and prognosis. Group 1: in which the main symptoms were somnolence and confusion accompanied by high fever, pathological C. S. F. and always abnormal EEG. The prognosis in this group was invariably bad. Group 2: with benign course, only slight pleocytosis, subfebrile temperature and practically normal EEG. The neurological symptoms in this group might be: a) eye- muscle paresis only, b) cases with cerebellar symptoms, c) cases with unilateral symptoms with or without peripheral cranial nerve paresis, d) cases with extrapyramidal symptoms and finally e) cases with midbrain symptoms. Four cases were fatal, but the authors do not mention the pathology.

In the second half of 1958 several reports on neurological complications in Asian influenza have been published in England. Well-documented cases have thus been presented by Mc Conkey & Daws (13), Compton Smith (17), Mc Gill & Goodbody (14) and Dunbar & al. (7).

Attention has been drawn to the fact that cerebral manifestations in Asian influenza may present themselves as psychotic outbursts (4, 18). The authors think that the finding of grossly abnormal EEG justify the view that these psychoses are symptoms of encephalitis and do not merely represent latent psychoses which have become manifest during a generalized infection.

In the communication of Flewett & Hoult (8) ten cases of influenzal encephalitis are reported together with two patients presenting the clinical picture of a polyradiculitis. A special group of patients present a history of an influenza-like infection followed after an interval of a few days by neurological illness and in which the serological reactions for influenza remained persistently negative. These cases are therefore considered as misleading.

Hermann & al. (11) mention the neuropathological findings in a nine months old boy, whose brain showed signs of meningitis, and in two other children a cerebral edema was found. Histological examination does not seem to have been performed. Of Giles & Shuttleworth's cases two presented clinical signs of encephalitis. In both cases the brains were found markedly congested, rather edematous, but microscopical examination failed to show evidence of virus encephalitis.

In this country as yet no reports on neurological complications in the Asian influenza epidemic have appeared. We have therefore thought it of some interest to present the clinical features of our case material.

During the fall of 1957 and the early spring of 1958 we have seen 12 patients presenting a variety of cerebral affections occurring in close relation to infections with Asian influenza. Four patients were children under ten years, two girls and two boys, the other patients were from 17—57 years of age, two women and six men. In five patients the first neurological symptoms occurred concomitantly with the influenza infection, in the rest of the patients a few days after the symptoms of general infection had subsided.

In the following the case histories will be presented. There was one fatal case, which will be described first.

CASE HISTORIES

Case No. 1.

Fourteen-month-old girl who fell ill at the same time as her three sisters and brothers with symptoms of influenza, fever of 39—40° C, and vomiting. In the course of a few hours, she had several universal epileptic seizures with unconsciousness and cyanosis. After admission to a local hospital she quickly became drowsy, rigid, shuddering with several epileptic seizures in spite of sedatives. Spinal fluid contained 13 lymphocytes per mm^3, and 15 mg per cent protein. Spinal fluid sugar 71 mg per cent. W. B. C. 8440.

At admission to this department she was seriously ill, pale, slightly cyanotic, deeply unconscious with extension spasms of all extremities which were rigid. Moist rales were heard over both lungs. In spite of treatment with erythromycin, penicillin, hydrocortisone and gammaglobulin the condition rapidly deteriorated and the patient died 19 hours after admission and 4½ days after the first symptoms. Autopsy was not allowed.

We feel that the primary factors in the clinical picture were the severe influenza infection with a severe pneumonia and that the neurological symptoms should be interpreted as a toxic encephalopathy rather than an encephalitis *sensu strictiori.*

The remaining 11 patients presented all light to moderately severe cases.

The next patient developed a Parkinsonism in the first months following an influenza infection, which in the beginning had dizziness as a dominating symptom.

Case No. 2.

Fifty-seven-year-old man with a negative past history. November 1957 influenza with fever for a couple of days, during which he had difficulty in sleeping. Since then he was abnormally tired, developed stiffness of movements in all extremities and subsequently progressive coarse tremor of the hands and some difficulty in concentrating. At the admission here nine months after the initial symptoms he was found oligomimic and mentally somewhat sluggish. There was nystagmus and tremor of the tongue. Moderate rigidity of all extremities and tremor of the hands. The deep reflexes were somewhat exaggerated on the right extremities, and a positive Babinski sign was present on the right.

Examination of vestibular reactions: nystagmus of central origin. Pneumography: slight symmetrical dilatation of the ventricular system. Spinal fluid: seven lymphocytes per mm³, total protein: 58 mg per cent. EEG: moderate — severely abnormal with 4—7 c/s activity and sharp waves bitemporally and predominance on the left. The condition was somewhat bettered by antiparkinsonian drugs.

Case No. 3.

Forty-year-old man who in the last days of November 1957 fell ill with general dedolations, headache and slight fever lasting for a few days. A couple of days after normalization of temperature he suddenly felt blurring of vision in the right eye and at the same time it was noted that the right pupil was dilated. The condition has remained unchanged since. At the examination here five months later, the right pupil was found widely dilated with no direct or consensual reaction to light, but otherwise no neurological abnormalities were found. Spinal fluid normal. EEG normal.

We think that the pathological substratum of the internal ophthalmoplegia in this case must have been a localized mesencephalic encephalitis.

The last nine patients we have divided in two clinical groups — one comprising four patients with predominant cerebellar symptoms and the other comprising five patients with epilepsy as the main symptom.

Case No. 4.

Twenty-year-old soldier who 14 days before admission fell ill at the same time as many of his fellow-soldiers with symptoms of influenza. He lay in bed for six days, but had no fever after three days. Two days later he became very dizzy with unsteadiness on walking, diffuse headaches and dysarthria together with slight fever, vomiting and blurring of vision, but no mental clouding. At the admission he was euphoric, but mentally clear. Speech was very dysarthric. There was slight paresis of the velum of the soft palate on the left. Nystagmoid jerks on looking to the left. No paresis or disorder of tone of extremities, but preponderance to the right of the deep reflexes and marked ataxia of all extremities. The gait was rather atactic.

W. B. C. 12200—9000; normal differential count.

Spinal fluid six lymphocytes per mm³, total protein: 78 mg per cent.

No bacteria on inoculation.

Vestibular examination: spontaneous nystagmus to the left.

Eight days later he had no complaints, he was less euphoric, speech only slightly dysarthric, and there was only minimal ataxia of the leftsided extremities. Two months later there was only slight nystagmus to the left and minimal ataxia of the left leg.

Case No. 5.

Forty-one-year-old man. November 1957 influenza — was confined to bed for 14 days with high fever, severe headache, somnolence and severe dizziness. Since then gyratoric dizziness, nausea and vomiting provoked by movements of the head and accompanied by permanent tiredness and intermittent blurring of vision.

Here we found nystagmus, rightsided central facial palsy, very lively deep reflexes, and the gait showed instability in turnings.

Vestibular examination: nystagmus of central origin.

EEG: slight nonspecific dysrythmia.

Case No. 6.

Ten-year-old boy, who at the end of January 1958 had symptoms of influenza like the rest of the family. On February 5, he complained of dizziness and headache. Since then several times weekly acute gyratoric dizziness, atactic gait, blurring of vision and throbbing frontal headache lasting for hours.

At the admission in March 1958 he presented ataxia of both upper extremities and bilateral dysdiadochokinesis. Spinal fluid was normal. Vestibular reactions normal.

Case No. 7.

Thirty-year-old woman, who had had migrainous headache since the age of 16. In October 1956 she was examined in our department, but nothing abnormal was found at clinical examination. EEG was abnormal on photostimulation with square waves in both occipital regions. After treatment with dilantine the frequency of the headache was greatly reduced. In the beginning of November 1957 the patient had a sore throat, dry cough and slight fever. Since then increasing vertigo with nausea and vomiting. During a subsequent admission to a local hospital she was mentally clouded for a couple of days and had difficulty in steering her arms. A horizontal nystagmus was observed. Spinal fluid normal. At the admission here six weeks after the initial symptoms the neurological findings were: lively spontaneous nystagmus, slight terminal ataxia of right arm and of both legs. EEG was still abnormal, paroxystic during photostimulation. Vestibular examination: nystagmus of central origin. During her stay in hospital she gradually recovered, and control examination three months later in the out-patient department was quite normal.

Summary of Group I: the dominant subjective symptom was vertigo arising in the last days of an influenza infection or a few days after having recovered from the infection. In two patients there were furthermore complaints of difficulty in steering the extremities, and one had dysarthria. Clinical examination revealed nystagmus in three patients, which proved to be of central origin by the vestibular examination. In three patients slight ataxia was present, while a fourth beside ataxia had dysarthria and slight unilateral signs of pyramidal tract lesion.

The second clinical group comprises five patients who developed epileptic phenomena — one during the acute phase of the infection, the others a few days after the symptoms of infection had disappeared.

Case No. 8.

Thirty-seven-year-old man. Past history negative. In the days December 5—9, 1957 symptoms of influenza with marked elevation of temperature and headache. On Dec. 11, in the evening he became drowsy, had nausea and vomited. Dec. 12, short-lasting

universal convulsions, after which he was mentally clouded, agitated and restless, did not sleep during the admission to a local hospital for two days. He was then transferred to a mental hospital for five months. In the beginning he was confused, disoriented, had hallucinations.

Gradual improvement followed. Spinal fluid and EEG normal.

One month after discharge from the mental hospital he had six seizures with universal convulsions, involuntary excretion and biting of the tongue preceded by mental confusion and complaint of smelling odd smells for one to two hours.

On admission here August 1958 clinical examination revealed: mentally sluggish, somewhat oligomimic. Deep reflexes exaggerated on the right side. Vestibular examination: nystagmus of central origin. Pneumography: moderate cortical and ventricular atrophy. Left carotid arteriography: normal.

EEG slightly abnormal. During hyperventilation and photostimulation moderately abnormal with 3—5 c/s activity frontotemporal and slight preponderance over left temporal.

Spinal fluid six lymphocytes per mm^3, total protein: 47 mg per cent.

The patient was treated with Dilantine 200 mg × 2 and had no seizures during the observation period.

Case No. 9.

Eight-year-old boy. January 1958 the parents had symptoms of influenza. Two days later the patient complained of headache, had nausea and vomiting and for the following days petit mal seizures and confusional states lasting about one hour. On January 10, he became unconscious with deviation of gaze to the right and one hour later universal convulsions lasting four hours. He was admitted to the Neurosurgical Department of the University Hospital, Copenhagen, and was here found to be mentally clear with jerks in left facial muscles and the left arm. The following days he was somnolent with aphasia and the described clonic jerks. EEG was severely abnormal with focal changes in the right temporal lobe. Right-sided carotid angiography and ventriculography were normal, and the same applies to repeated spinal fluid examinations. He was transferred to our department and now there was only a slight leftsided preponderance of the deep reflexes, but otherwise normal neurological findings. EEG severely abnormal with 2—3 c/s activity universal, slight preponderance of the changes to the left. During antispasmodic treatment no seizures were seen.

Case No. 10.

Seventeen-year-old man who from November 5—11 had symptoms of influenza. The day after having left hospital he felt tired, drowsy and a few hours later had an epileptic seizure with unconsciousness, universal convulsions and involuntary excretion after which he was mentally confused for about 24 hours. He was admitted to a local hospital, where spinal fluid contained 21 lymphocytes per mm^3 and normal protein. He has since complained of failing memory. Clinical examination December 1957 showed weakened right abdominal reflexes and exaggeration of the rightsided deep reflexes, but no Babinski sign. EEG severely abnormal, paroxystic during photostimulation. He was treated with dilantine 100 mg three times a day and at a control examination five months later nothing abnormal was found at neurological examination, but EEG was unchanged. He had a couple of months earlier had one epileptic seizure after not having taken his medicine for some days, otherwise no epileptic manifestations.

Case No. 11.

Twenty-five-year-old woman who October 1957 had symptoms of influenza with high fever for nine days, headache, and somnolence. Since then she had short-lasting fits during which she felt unreally big and the room correspondingly big, and she had several fits during which she felt in a dreamy state. May 1958 three universal epileptic seizures.

Nothing abnormal was found at clinical examination. EEG: severely abnormal with bitemporal small spikes and 3—5 c/s activity and with the greatest amplitude over right hemisphere.

Pneumography: normal ventricular system. Spinal fluid: 12 lymphocytes per mm^3, total protein: 33 mg per cent.

During her stay in hospital she had one seizure with universal tonic and clonic convulsions and unconsciousness. After treatment with mysoline no seizures were seen.

Case No. 12.

Four-year-old girl. Past history negative. December 1957 symptoms of influenza like the rest of the family. She had moderate elevation of temperature, which was normalized January 3. During that day two universal epileptic seizures occurred. On January 6, again an epileptic seizure, and during subsequent admission to a local hospital she was somnolent and subfebrile for 48 hours. She was transferred to this department on account of several grand mal seizures.

We found her pale with exaggerated deep reflexes and a Babinski sign on the right side. EEG severely abnormal with 1—3 c/s activity universally, no focal abnormalities. W. B. C. 12500—9000. X-ray photo of skull normal.

During her stay in hospital numerous grand mal and petit mal seizures were observed in spite of antiepileptic treatment.

Summary of Group II: all five patients developed epileptic phenomena in close relationship to influenza infection. All had epilepsy of grand mal type, one furthermore focal seizures in the initial phase of the disease, one petit mal seizures and in two patients also psychomotor phenomena were seen. One patient, coming to observation six months after the acute illness, presented no neurological abnormalities, the remaining four patients showed slight signs of unilateral pyramidal tract lesion. All patients had abnormal EEG. In none of the patients there was a family history of epilepsy nor traumatic lesions of the central nervous system in the anamnesis.

DISCUSSION AND CONCLUSION

In one case the nervous symptoms were interpreted as the result of a toxic encephalopathy and not as an encephalitis proper. In the remaining cases the signs and symptoms suggested

an encephalitis, and the close relation in time between the influenza and the cerebral symptoms indicated an influenza virus of the Asian strain to be the causative agent, although final serological proof or isolation of the influenza virus is lacking.

While the prognosis in the first group of patients with cerebellar symptoms seems to be good, it is more uncertain in the group of patients with epileptic seizures. The presumed predominant cortical cerebral damage in these cases is probably irreparable. In one patient the epileptic seizures continued in spite of intensive antiepileptic treatment, in the others no seizures were seen during the observation period, but this is still too short to allow any final evaluation of the prognosis in these cases.

As earlier stressed it is presumably not permissible to take the relatively great number of patients with encephalitis seen over a short period as an expression of a special neurotropicity of the Asian strain of influenza virus. It rather reflects the fact that the influenza infection has been almost ubiquitous with a subsequent greater possibility of central nervous system affection.

The purpose of reporting our cases has been to point out the fact that probably neuroinfections have been rather common during the recent Asian influenza epidemic and that the possibility of sequels to neuroinfections with Asian influenza virus should be borne in mind when clinical neurological pictures otherwise unexplained are encountered in the years to come.

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REMISSION IN CHRONIC MYELOID LEUCÆMIA FOLLOWING PROLONGED NITROUS OXIDE INHALATION

By H. C. A. LASSEN and H. SUND KRISTENSEN

In 1953 Bjørneboe et al. (1954) introduced the prolonged use of nitrous oxide anæsthesia in the treatment of severe tetanus. Lassen et al. (1954) described four such cases with one death. This patient, a boy of 15, seemed on the road to recovery, when, on the 18th day at a point where the tetanus had practically subsided he developed signs of acute aplastic anæmia with pronounced granulocytopenia, thrombocytopenia and severe hæmorrhagic diathesis, followed by septicæmia due to Escherichia coli and Pseudomonas pyocyanea, from which the boy succumbed ten days later.

When re-examining the case-histories of the first two patients, who had recovered, it became clear that they also had shown signs of hæmatological complications.

From the Blegdam Hospital, Copenhagen.

Gormsen (1955) published a case of severe tetanus showing agranulocytosis and thrombocytopenia in a boy treated with prolonged nitrous oxide anæsthesia, chlorpromazine and curare. He presumed that the hæmatological changes were due to the large doses of curare. Similar cases were described by Last & Nicholas (1956) and Wilson et al. (1956).

The same year (1956) we published an analysis of 13 severe cases of tetanus in order to find the cause of the acute bone-marrow depression, and against our expectations it seemed probable that the acute aplastic anæmia was due to the prolonged nitrous oxide anæsthesia. This was proved beyond all reasonable doubt by two clinical trials.

Since then we have performed numerous animal experiments without being able to reproduce our observations in patients with severe tetanus treated with prolonged nitrous oxide anæsthesia.

There were certain indications, however, of a cytotoxic effect on embryonic tissues. Kieler (1957) in tissue-cultures of embryonic mouse-heart myoblasts kept in a 50 per cent atmosphere of nitrous oxide found a pronounced cytotoxic effect of the anæsthetic.

As the acute bone-marrow depression in patients with tetanus had proved easily reversible when N_2O was discontinued, we decided to investigate the effect of the anæsthetic in cases of chronic myeloid leucæmia.

In the following the case-histories of two such patients treated with nitrous oxide inhalation are described. The first patient had one course of continued N_2O treatment, the second patient three separate courses.

In both cases a polyethylene catheter was introduced through the nose and a mixture of four litres of commercial nitrous oxide and one litre of oxygen per minute was blown into the nasopharynx. In order to avoid desiccation of the mucous membranes of the respiratory passages the mixture was first passed through an electric humidifier. The percentage of nitrous oxide in the alveolar air under the conditions described was calculated to be about 25 per cent.

Case No. 1. A woman of 59 was admitted on June 24, 1958, with a history of night-sweats for two years, increasing lassitude, anorexia and mild diabetes. The temperature was 39° C, the spleen was down to the umbilical level. Examination of the blood showed pronounced anæmia, high thrombocyte counts and white cell counts around 200,000 per mm^3 with a high percentage of immature cells (Fig. 1). Bone-marrow biopsy confirmed the diagnosis of chronic myeloid leucæmia. Analysis of vitamin B_{12} in the blood on the 11th day in hospital showed a content of no less than 4900 $\mu\mu$ grams per ml (normal values for adults between 200—900 $\mu\mu$ grams per ml). The following day nitrous oxide inhalation was started. The patient had no specific complains during the treatment. She became euphoric, verbose and somewhat confused as if she were subject to a light alcoholic intoxication.

On the fifth day of the treatment the bone-marrow showed severe atypical granulopoiesis resembling pernicious anæmia. The B_{12} content had now come down to 3100 $\mu\mu$ grams per ml.

On the 10th day of the treatment bone-marrow examination showed the erythropoiesis to be frankly myeloblastic. After a few days of treatment the number of myeloid cells began to decrease and reached a minimum of 54,000 on the 13th day. The mononuclear white cells remained unchanged. At the same time the thrombocytes came down from well over 1 million to about half a million, and the B_{12} content on the 10th day of treatment fell to 2360 $\mu\mu$ grams per ml.

On July 19 the treatment was discontinued and a few days later the white cell counts began to rise reaching 130,000 on July 24.

Immediately after the withdrawal of nitrous oxide the patient's condition began to deteriorate, she became cyanotic, and dyspnoeic and her blood-sugar began to rise. Pulmonary embolism was suspected and hyperthermia and irreversible vascular shock developed.

Autopsy showed extensive pulmonary thrombosis and a greatly enlarged spleen. No bacterial growth was obtained from the heart, brain, lungs of the spleen.

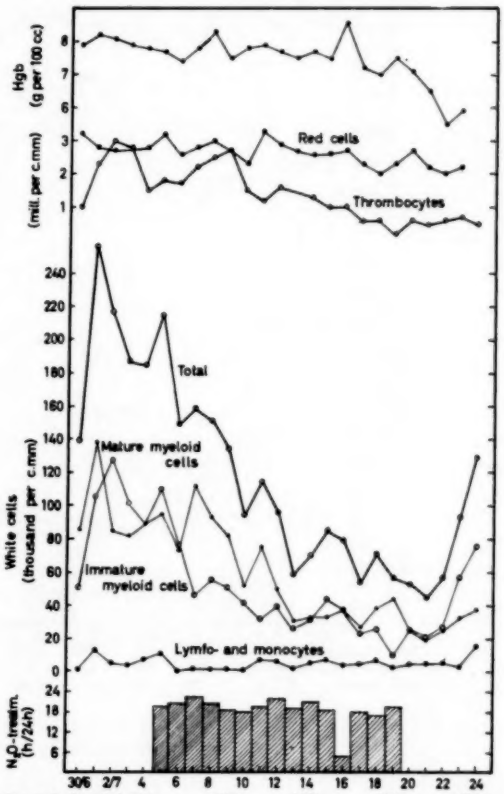

Fig. 1.
Blood changes in patient number 1.

Case No. 2. A man of 49 was admitted on June 20, 1958, with a diagnosis of splenomegaly. Two months earlier he started to feel feverish, he complained of abdominal distension, his appetite became poor and his weight decreased sharply. At admission, however, his general condition was quite good. The liver was palpable just below the costal margin and the spleen reached down into the left lower quadrant. Examination of the blood showed pronounced anæmia, normal thrombocyte counts and 40—90,000 white cells per mm^3 with a high percentage of myeloid cells (Fig. 2). Bone-marrow examination confirmed the diagnosis of chronic myeloid leucæmia. On July 4 the B_{12} content of the blood was 8500 $\mu\mu$ grams per ml. The next day nitrous-oxide inhalation was started in the way previously described.

The hæmatologic response is shown in Fig. 2. It was in every respect identical with the response observed in the first patient. A few days after the discontinuance of the anæsthetic the myeloid cells and

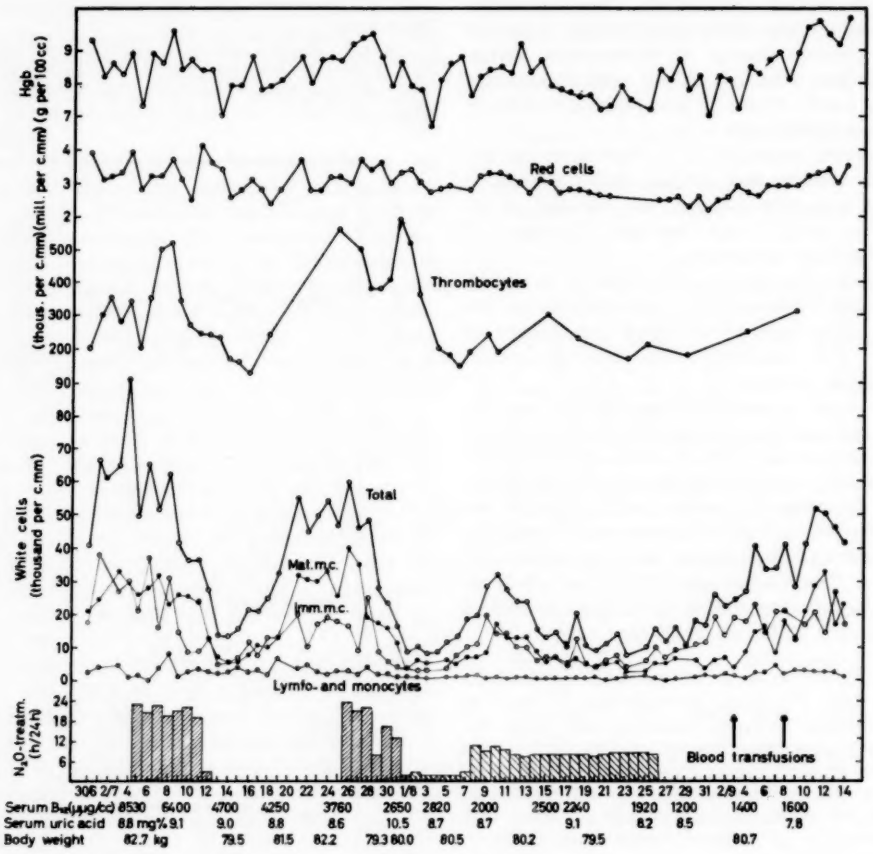

Fig. 2.
Blood changes in patient number 2.

the thrombocytes began to rise. A renewed course of nitrous-oxide starting on July 25 was followed shortly after by a sharp remission affecting the thrombocytes as well as the leucocytes. The patient was now placed on a "maintenance dose" of 2 litres of nitrous oxide and one half litre of oxygen per minute for 8—12 hours per day on which dosis the leucocyte counts were slightly above normal, although the percentage of immature cells remained high. During this rather prolonged period of treatment the condition of the patient deteriorated, he became weak, mentally depressed and slightly confused. The size of the spleen was not affected. After discontinuing the anæsthetic and two blood transfusions his general condition rapidly became better. At the same time the leucocytes increased to about the pre-medication level.

During the treatment bone-marrow examinations showed a histologic picture resembling pernicious anæmia. He left the hospital on August 19.

Two months later he was readmitted. The anæmia was pronounced and the leucocytes up to 70—80,000 per cubic mm with a high percentage of immature cells.

A third course of nitrous oxide inhalation (three liters of N_2O + 0.75 liter of O_2 per minute) was now started (Fig. 3), followed by a remission similar to those observed previously. At the same time, however, his general condition became worse, signs of pneumonia and vascular shock developed, and the anæsthetic had to be discontinued. Repeated blood-transfusions, large doses of penicillin and cortisone, however, rapidly restored his general condition. At the same time the number of white cells increased considerably.

SUMMARY AND CONCLUSION

Prolonged inhalation of nitrous oxide in two patients with chronic myeloid leucæmia had the following effects:

1. The total number of myeloid white cells and thrombocytes decreased considerably after five to 15 days of treatment and rose rapidly a few days after withdrawal of the anæsthetic.
2. The erythrocytes and hæmoglobin content were not affected.
3. Under the treatment the granulopoieses changed in a way resembling the bone-marrow picture seen in pernicious anæmia.
4. It has been known for some time (Beard et al. (1954), Mollin and Ross (1955))

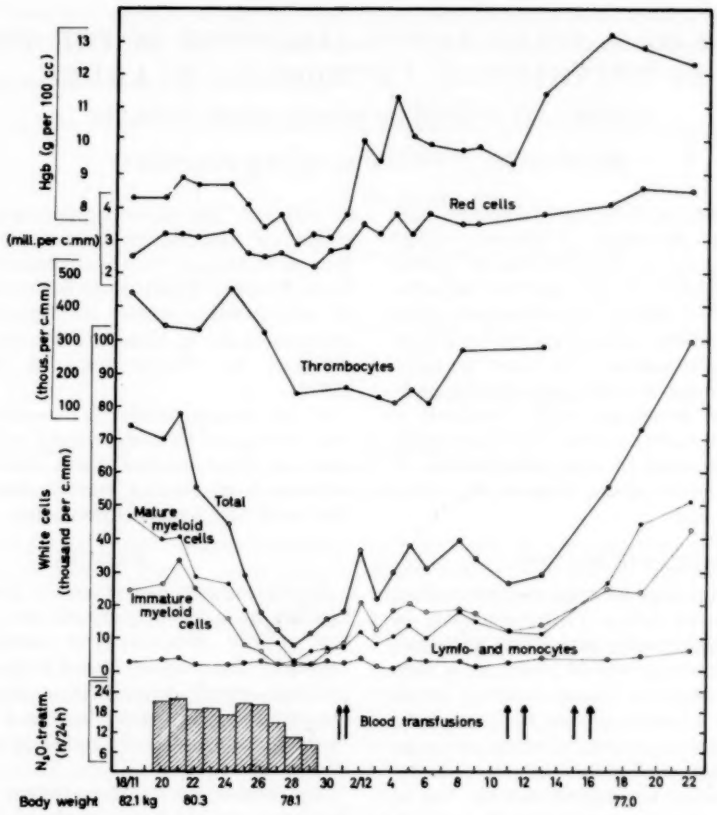

Fig. 3.
Blood changes in patient number 2.

that the content of vitamin B_{12} in the blood of patients with chronic myeloid leucæmia is considerably above normal. The cause of this is not clear, but Mollin et al. (1956) found that patients with chronic myeloid leucæmia retained vitamin B_{12} far in excess of the normal. Mendelsohn et al. (1958) showed this to be due to an increased content of a B_{12}-binding protein in the α_1 fraction of the serum globulin. During the treatment with nitrous oxide a sharp fall in the B_{12} content was observed, although normal values were not reached. This fall started early followed by a rapid increase after discontinuation of the anæsthetic.

5. During the four courses of treatment certain changes in the patients' mental state were noticed. In the beginning they felt like being under the influence of alcohol, but later on they complained of mental depression, extreme lassitude and on two occasions even stupor or coma ensued.

In conclusion we want to emphasise that prolonged inhalation of nitrous oxide in the way we have used it is not to be recommended as a therapeutic agent in cases of chronic myeloid leucæmia. We feel, however, that from a theoretical point of view these cases are of interest in stressing the fact that nitrous oxide has a marked cyto-toxic effect on the bone-marrow when used over prolonged periods of time.

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THE EFFECT OF A POLYSYNAPTIC INHIBITOR IN THE TREATMENT OF UNINHIBITED NEUROGENIC BLADDER

CYSTOMETRIC STUDIES ON TWENTY-EIGHT PATIENTS

By PALLE JUUL-JENSEN and EJNER PEDERSEN

During the treatment of spastic paraplegia with the polysynaptic inhibitor *Lisidonil* (CIBA 13.155), Pedersen & Schleisner (1959) observed — in addition to the desired reduction in muscular tone — clinical improvement in the disturbances in bladder control which were present in some of their patients. In order to assess this effect of the drug, we have subjected a series of patients under treatment with Lisidonil to cystometric determinations, since such determinations must be assumed to give quantitative information of the effect of the drug on the spinal reflex activity in man.

MATERIAL AND METHODS

The series comprised 28 patients with disseminated sclerosis, of whom 16 were greatly incapacitated and apparently in a fairly stationary phase, while the remaining 12 were in a more labile and remittent phase. Theoretically, it should be expected that a beneficial effect, if any, of a polysynaptic inhibitor might be obtained in patients with frequent and urgent micturition, and as this was rapidly confirmed during the investigation, the series was selected with a special view to this type of bladder dysfunction. For the inclusion of a patient in the series is was required that Lisidonil treatment had been given for a reasonably long period, and that cystometry was performed before and during the course of treatment.

Cystometry was performed by the technique described by Povlsen (1941), in which the bladder is filled through a urethral catheter by slow injection of sterile water and the intravesical pressure measured on a connected manometer for each 50 ml injected. In order to minimise the risk of urinary tract infection ammonium chloride therapy was given a few days before and after the cystometric determinations.

Chemically, Lisidonil is 2-hydrazino-4,6-bis-diethyl-amino-1,3,5-triazine D-tartrate (in the previous study, the chloride compound was used; its effect is similar to that of the tartrate, but is about 1.25 times as great per weight unit). In animal experiments, the drug has shown a slightly inhibitory effect on spinal reflex activity, more pronounced on multisynaptic than on monosynaptic reflexes. The experiments failed to reveal any action on neuromuscular transmission, and the drug scarcely exerts any influence on the cerebrum. Further details as to the action of the drug in animal experiments, its depressive effect on muscular tone in man and its side effects were reported by Pedersen & Schleisner (1959).

In the present study, the average dosage level was 16 mg per kg body weight in 24 hours given orally in three divided doses. On an average, the patients were treated for 30 days between the first and the second cystometric determination.

RESULTS

In the clinical evaluation of bladder dysfunction the 28 patients were divided as follows: 24 had mainly frequent and urgent micturition (many of these also suffered from incontinence); two had mainly difficulties in micturition, while the last two were described as a "mixed type", as they could not be included in one of the first two groups.

The evaluation of the clinical effect of Lisidonil was chiefly based on the observations made by the nursing staff and only to a minor extent on the information given by the patients. Clinical improvement was obtained in 22 of the 28 patients, evidenced by longer intervals between the micturitions, diminution of the urgency, and a change from incontinence to continence. The greatest clinical improvement was obtained in patients who had not yet been completely incapacitated by their disease. These patients could be rendered almost completely symptom-free, while the effect in patients with so extensive changes that they functionally had a "reflex neurogenic bladder" was manifested merely by longer intervals between the urinations. However, even such a moderate clinical change may improve the well-being of the patients and ease the work of the nursing staff.

Table 1 shows the clinical and cystometric changes observed in the 28 patients during the treatment with Lisidonil.

In the 24 patients with frequent and urgent micturition, the cystometrograms obtained before treatment were of the "uninhibited neurogenic" type in 21, while they were hypertonic in the remaining three. During the treatment, the cystometrograms approached normal in 22 of the 24 patients. In 11 patients, the changes were moderate (Figs. 1 and 2), and in patients with changes

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Lisidonil was supplied by CIBA Ltd., Basle.

Table 1.

Clinical and cystometric changes in 28 patients with disseminated sclerosis under treatment with Lisidonil.

Type of bladder dysfunction	Clinical effect	CYSTOMETROGRAMS	
		Bladder type before treatment	Change after treatment
Urgent urination (24)	Yes (20)	Uninhibited neurogenic (19) ..	Moderate (9)
			Distinct (10)
		Hypertonic (1)	None (1)
	No (4)	Uninhibited neurogenic (2) ..	Moderate (1)
			Distinct (1)
		Hypertonic (2)	Moderate (1)
			None (1)
"Mixed type" (2)	Yes (2)	Uninhibited neurogenic (2) ..	Moderate (1)
			None (1)
Difficulties in urination (2) ..	No (2)	Hypotonic (2)	None (2)

The figures in parentheses indicate the number of patients in the various groups.

as those shown in figure 1, the clinical improvement was evidenced only by longer intervals between the urinations. In the remaining 11 patients, distinct changes in the cystometrograms were observed (Figs. 3 and 4).

In the two patients who had difficulties in micturition, cystometry showed a hypotonic and dilated bladder; treatment with Lisidonil did not result in any change in the cystometrograms (Fig. 5) or in clinical improvement.

In the two patients who had been described clinically as "mixed type", the bladders were of the "uninhibited neurogenic" type; a clinical effect was obtained in both cases, and in one of them the cystometrogram approached normal.

In six patients, no residual urine was found at any of the two examinations, while 12 showed a slightly greater amount of residual urine at the second than at the first cystometric determination. Residual urine had decreased at the second determination in 10 patients.

The urine was analysed in all the patients; intravenous pyelography was done in a few cases, and all male patients were subjected to rectal examination, but these investigations did not reveal conditions which might be assumed to be of significance in the changes observed in the cystometrograms from the first to the second cystometric determination.

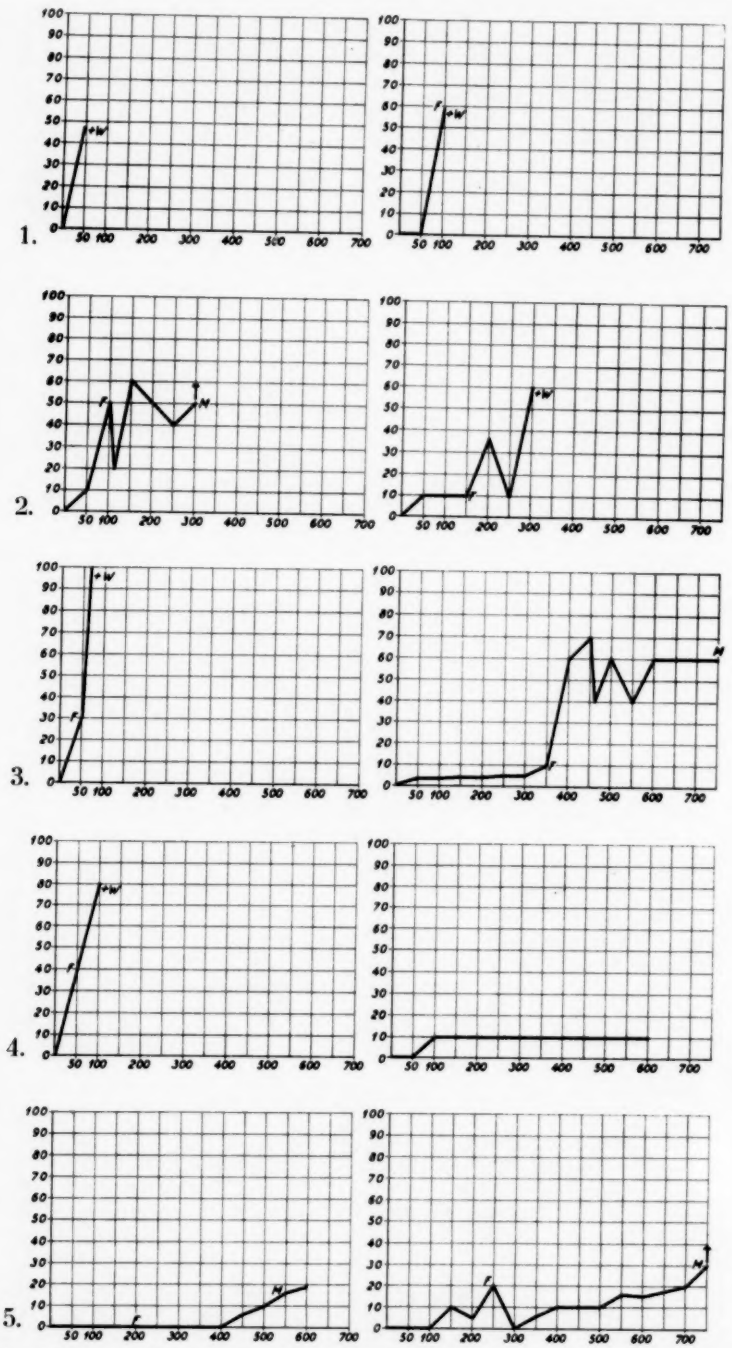
DISCUSSION

In the present study, attempts were made to obtain a quantitative impression of the effect of Lisidonil by means of cystometry, which gives a possibility of assessing the change in the reflex contraction of the bladder which may be elicited through the spinal cord by the distension of the bladder and the attendant stretching of the vesical muscles.

The method of cystometry which was employed is acceptable in a comparison of the cystometrograms before and during treatment with Lisidonil. The method of choice would undoubtedly have been excretory cystometry introduced by Comarr (1957), in which the patient drinks ample amounts of water, so that the bladder is filled in the normal way through the ureters, but this method, too, incorporates an unphysiological component, *viz.*, the insertion of a urethral catheter. This catheter may excite reflexes from the vesical mucosa, for example, resulting in contraction of the muscles of the pelvic floor (Bors & Blinn 1957). However, this phenomenon does not cause any systematic errors as the experimental conditions were uniform at all the determinations.

Our studies showed that Lisidonil affects the reflex response of the bladder muscles in a large proportion of patients in whom this reflex response is hypernormal, as in "uninhibited neurogenic bladder". The conditions must be assumed to be of the same character as is seen in spasticity of the extremital muscles, *viz.*, that loss of central inhibition results in a facilitation of the spinal reflex activity. The drug is effective only in that type of bladder dysfunction, and the best effect is obtained in cases where spasticity is predominant as compared with bladder paralysis and sensory disturbances.

The present series showed agreement between the clinical improvement and the changes in the cystometrograms. The patients were largely severely affected, some of them presumably with irreversible patho-anatomical changes limiting the therapeutic possibilities, but in many cases the results obtained will undoubtedly prove to be of value. The aforementioned average dosage level, 16 mg per kg body weight in 24 hours, is relatively high; in some cases, the dose was later slightly reduced because of distressing fatigue.



Figs. 1 and 2. — *Cystometrograms showing moderate changes during treatment with Lisidonil. The abscissa indicates the volume in ml and the ordinate the intravesical pressure in mm Hg.*
W, voiding around the catheter. F, first desire to micturate. M, painful distension of the bladder. The arrow indicates voluntary voiding contraction.

Figs. 3 and 4. — *Cystometrograms showing distinct changes during treatment with Lisidonil.*

Fig. 5. — *Cystometrograms showing hypotonic bladder without change during treatment with Lisidonil.*

In spite of the high dosage, no cases of urinary retention were encountered during the observation period in hospital, *i. e.*, the drug does not abolish the bladder spasms which in patients with bladder paralysis represent the only possibility of spontaneous urination. In one patient with severe brain-stem symptoms and dementia who was under treatment with 13 mg Lisidonil per kg body weight, difficulties in micturition developed three weeks after discharge; the difficulties diminished after withdrawal of the drug. Three days later the patient died from respiratory failure, probably due to progression of the brain-stem lesions.

Theoretically, it should be expected that inhibition of the spinal reflex activity of the bladder would tend to increase the volume of residual urine, but the cystometric determinations did not reveal definite evidence in support of this assumption.

The effect of Lisidonil is of an "atropine-like" character, similar to that obtained by Banthine (Lapides & Dodson 1953, Draper et al. 1953).

Apart from what has already been stated, the present study did not reveal additional side effects of the drug. Lisidonil tends to result in constipation, but is did not seem to affect cardiac or respiratory function, which Millefiorini & Donini (1958) reported to have observed in a few cases after intravenous administration of the drug.

Lisidonil was given in relatively large doses, but in routine treatment the initial dose should scarcely be higher than five to ten mg per kg, since sufficient experience in long-term treatment with appreciably higher doses is not yet available. The dosage level may then be adjusted in the individual cases.

SUMMARY

The effect of Lisidonil on bladder dysfunction was studied in 28 patients with disseminated sclerosis. The studies showed that the drug is effective in patients with a cystometrogram of the "uninhibited neurogenic" type, which is evidenced clinically by frequent and urgent micturition. In many of the patients micturition approached normal during treatment. In patients with a functional "reflex neurogenic bladder" the treatment resulted in longer intervals between the urinations. In routine treatment, the initial dose of Lisidonil should scarcely be higher than five to ten mg per kg body weight in 24 hours.

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SOME PROBLEMS IN CONNECTION WITH INTRATHECAL HYDROCORTISONE TREATMENT

By ERIK SKINHØJ and OLE BUUS

During recent years various reports on intrathecal hydrocortisone treatment of different neurological disorders have been published — so far of a preliminary nature — however rather auspicious.

The theoretical foundation for these therapeutical experiments — having been started independently of each other — has been the desire to profit by the anti-inflammatory qualities of the cortisone-group and the experience that it presumably was the blood-liquor barrier, which was to be blamed for the lack of clinical effect by general steroid treatment in conditions where such an effect theoretically should be expected.

Apparently, however, some of these fundamental problems ought to be solved, before the therapeutical enthusiasm gains ground.

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Amongst these unsolved questions are the following: —

(1) Is the blood-liquor barrier for steroids absolute or, if not, so massive that general treatment cannot produce concentrations of therapeutical value within the barrier?

(2) For how long a time does the steroid concentration remain at a therapeutical level in the subarachnoid space after a single intrathecal injection of a given dose?

(3) To how great an extent do steroids deposited by lumbar puncture equalise in the whole cerebrospinal space?

(4) Does intrathecal steroid treatment through lumbar puncture involve local or general risks?

With the aid of Bojesen's method for the determination of small amounts of hydrocortisone

in tissue fluids it should now be possible to answer these question.

METHOD

The principle in Bojesen's method consists in an esterification of the unknown amount of hydrocortisone with S^{35}-labelled pipsan (p-iodophenylsulphonic acid anhydride). The varying loss during the rest of the procedure is estimated by means of admixture of I^{131}-labelled pipsyl hydrocortisone so that it is possible by determining the ratio S^{35}/I^{131} to calculate the original amount of steroid if the relative specific activity is known. Within the range used the analysis has a standard deviation of about 0.45 μg per 100 ml, or for the high values about 4 per cent.

RESULTS

In a number of normal spinal fluids the hydrocortisone concentration has been determined as follows: 1.8, 1.2, 2.3, 2.7 and 7.0 μg per 100 ml.

For five days before the determination two out of the five subjects had received a total of 450 mg of meticorten for therapeutical purposes, and the values 1.8 μg per 100 ml and 2.7 μg per 100 ml were found in these two cases. These figures mean firstly, that a passage of hydrocortisone normally takes place to the subarachnoid space in concentrations corresponding to about 24 per cent of the concentration in the blood and, secondly that therapeutically active concentrations cannot normally be obtained within the blood-liquor barrier by peroral or parenteral treatment. The concept of "therapeutically active concentrations" is so far speculative, but the normal serum levels, which according to Bojesen's method have been found to be of the order of magnitude 10 μg per 100 ml, can presumably be considered a minimum. With regard to the effect of the blood-liquor barrier in the opposite direction, we can state that following intrathecal injection of 100 mg of hydrocortisone we were unable to demonstrate any change in the urinary excretion of 17-ketosteroids. Thus no passage of significant, let alone threatening, magnitude takes place.

After deposition of 100 mg of hydrocortisone acetate ("Hydrocortisat Leo") intrathecally in the lumbar region we have found the following concentrations at subsequent lumbar puncture in various subjects (Table 1).

The figures show that even three weeks after deposition of 100 mg, values are found in the spinal fluid which must be considered therapeutically active. It may be mentioned for comparison that a similar quantity deposited in a joint would have disappeared already after a few hours.

However, it is difficult to explain the obtained maximal values and the elimination curve with its practically constant values within the first week.

Table 1.

Concentration of hydrocortisone in the spinal fluid after deposition of 100 mg hydrocortisone acetate by lumbar puncture.

Time after injection	Concentration μg per 100 ml
1 hour	504
3 hours	575
24 "	496—417
7 days	526
10 "	342
12 "	328
13 "	157
14 "	23.5
21 "	21.5

We have found that the maximal solubility of hydrocortisone in spinal fluid at body temperature is 35 mg per 100 ml, *i. e.*, much higher than the highest concentrations found in our experiments, in spite of the fact that after deposition of the 100 mg of hydrocortisone acetate an initial concentration of at least 100 mg per 100 ml must be considered, and in spite of the fact that free hydrocortisone acetate could only be demonstrated spectrophotometrically in the sample taken one hour after deposition, whereas this examination was negative already in the 3-hour sample (*i. e.*, the concentration has then been <900 μg per 100 ml). To explain these apparently paradoxical findings it is necessary to assume a reversible structural binding of hydrocortisone acetate or hydrocortisone to the surrounding tissue.

We cannot know anything certain in advance as to whether a hydrocortisone acetate depot which is applied by lumbar puncture will mix throughout the subarachnoid space to such a degree that the cephalic concentration becomes sufficient. Twenty-four hours after deposition of 100 mg of hydrocortisone acetate in the lumbar region we have found by suboccipital puncture in unconscious patients concentrations of 76 μg per 100 ml and 98 μg pr 100 ml, and at ventricular puncture in the posterior horn 11.4 μg per 100 ml.

In no case of the now more than 100 lumbar injections of hydrocortisone acetate have we observed subjective or objective side-effects, except for a few of the ordinary complaints in connection with lumbar puncture, and in no case did we find pleocytosis or a rise in protein values indicative of local irritation on re-puncture. On the contrary, we have several times observed that an existing pleocytosis disappeared or decreased after deposition of hydrocortisone acetate. As it is not necessary either to consider the resorption of hydrocortisone acetate from the spinal space, the method seems to be practically free from risks.

SUMMARY

The spinal fluid in man contains hydrocortisone (compound F) in concentrations from 1.2 to 7 μg per 100 ml, *i. e.*, corresponding to about

20 per cent of the blood values. Owing to the blood-liquor barrier, therapeutically active concentrations in the intrathecal space cannot be obtained by peroral or parenteral treatment.

Intrathecal injection of hydrocortisone acetate does not seem to involve any risks, and following deposition of 100 mg by lumbar puncture a therapeutically active concentration in the spinal fluid of at least 3 weeks' duration is obtained.

Aided by a grant from Leo Pharmaceutical Products Trading Ltd.

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DELIRIUM TREMENS AND ITS TREATMENT

By BENT FLORIAN SØRENSEN

In the psychiatric literature, delirium tremens and its treatment are still subjects of considerable discussion.

Most authors agree (1) in the statement that vitamin-B deficiency predisposes to an attack of delirium tremens, and that acute somatic disease precipitates (or aggravates) such an attack, while abuse of alcohol is the pathogenetic factor. However, we do not know so much of the disease that a causal therapy in the individual case can be outlined. The forms of treatment which have been used vary within wide limits, and so do the results obtained. Although this is not surprising, the differences are sometimes so great that we must ask ourselves if the series of patients described in the literature are at all comparable. When the therapeutic effects of the same drug given in the same doses vary, it is reasonable, especially in a disorder like delirium tremens, to ask if the patients have been treated in the same stage of the disease. Often it is impossible to provide answers to these questions, and we must then content ourselves by ascertaining that some hospitals are satisfied with a mortality, the size of which would be disgraceful if it applied to the type of patients with whom we are concerned.

Viewed against this background, it has been thought that it would be of interest to give a description of delirium tremens and its treatment as it is given in the Psychiatric Hospital in Risskov (and presumably, in most other hospitals in Denmark).

INCIDENCE AND MORTALITY

Thanks to appropriate social measures against the abuse of alcohol, especially in the form of heavy taxation, the disease has become relatively rare in Denmark. During the years 1910 to 1914, the Psychiatric Department of the Municipal Hospital of Copenhagen, in which the majority of the cases of delirium tremens occurring in the Danish capital was treated, received about three hundred patients with this disease annually (2). In 1932, the number had decreased to eight cases. During the ten-year period from 1931 to 1940, the average number of cases per year was eighteen for the whole country. During the Second World War the annual average number decreased to eleven, but after 1945 it has again increased. Thus, thirty-one cases occurred in 1952, while twenty-one to twenty-five cases were reported annually from 1953 to 1955. The number of deaths due to delirium tremens was four in 1953, three in 1954, and two in 1955.

During the last two years the number of cases of delirium tremens seems to have been increasing (3).

In our hospital, we have during the last twenty years on an average encountered only one case annually, with only one death during the whole period. In the patient who died, the disease had been diagnosed at a very late stage and had partially been given erroneous treatment. Thus, in our hospital, as in other special departments in Denmark, the mortality from delirium tremens is extremely low. Usually, we take it for granted that we can save the life of a patient who is admitted with this disease.

From the above account it appears that the disease and its treatment do not give rise to great difficulties in psychiatric departments in this country. In my opinion, the explanation is that attention has, in particular, been focused on: —

1. Early recognition of delirium tremens or of the danger of its development in a given patient.

2. Early intensive veronal (diaethyl barbituric acid) treatment given in the actual cases — and, as a prophylactic measure, to patients in the "danger zone" — accompanied by adequate treatment of co-existing somatic disease, if any.

SYMPTOMS

While, thanks to the classic descriptions of the florid symptomatology of fully developed delirium, our knowledge of this phase of the dis-

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ease is extensive, its premonitory symptoms are often less well-known outside the circles of psychiatrists. As it is of crucial importance for the prognosis that the patient is treated as early as possible, I shall here consider the clinical features of the early stages of the disease in some detail, since an account of these is missing in most standard textbooks.

The symptoms foreshadowing that a patient is in the danger zone, or that delirium is actually developing, are especially sleep disturbances with awakening in anxiety, often because of terrifying dreams in which animals, often of fantastic shape, may be present; a feeling of pronounced inner unrest; restlessness; anxiety; and increasingly severe tremors in the chronic alcoholic. These symptoms may give rise to bouts of heavy drinking, which, however, only further aggravate the picture. The unrest and anxiety intensify, and pre-existing gastro-intestinal disorders often increase in severity. Visual hallucinations may occur when the patient turns off the light in order to go to sleep. The patient often sees animals, usually diminutive figures which are present in large numbers and, most frequently, moving. — Thus, one of our patients saw a large number of small pigs; others see lions, wolves or snakes in diminished size. In some patients, the visual hallucinations consist in small human beings or more impersonal objects, such as numerous small wooden shoes, etc., *i. e.*, a wide variation of mainly terrifying visions. It is characteristic of this phase of the disease that the patients are aware that these experiences are symptoms of a disease. The anxiety changes into frank fear; the patients dare not go to sleep, or even shut their eyes. They are accessible to transitory reassurance in conversations with the surroundings about something else. They are often disinclined to mention their hypnagogic hallucinations to others, and they do not always mention them to the doctor unless they are directly questioned about such hallucinations. Many dare not mention these experiences until they have got rid of them. At this stage, the patients are fully oriented as to time, place and personal data. Many of the patients go to work the next day, and go on drinking, but in the evening the hypnagogic hallucinations recur. The patients feel that the plot thickens; a few realize what all this means and apply for medical aid, or they may be able to abstain from alcohol for a certain length of time. Others continue their drinking habits, or even indulge further in alcohol, which leads to incipient delirium. The hypnagogic hallucinations may now become incorrigible. — One evening when the light was turned off, a man who was later admitted to this hospital saw lions which attacked him. He began to fight with them and believed that he had torn off a leg from one of the animals. Later he saw small white rabbits with red eyes. When he stretched his arm to reach them, they receded into the background. These terrifying hallucinations disappeared when the light was turned on. He had previously had delirium tremens and felt a new attack was developing, for which reason he applied to his doctor. On admission, he was fully oriented, also as to time and place.

When the condition, as is often the case, is precipitated by acute somatic disease, such as pneumonia, severe gastritis, fracture of a limb or head injuries, the patient passes rapidly, often within a few hours, into the state of fully developed delirium. This stage is characterized by a more or less complete disorientation as to time, place, surroundings and the whole situation, while the patient's memory for his name, personal data and family conditions is retained. The patient believes he is in his own town, in his usual environment, and mistakes those surrounding him for old acquaintances. Occupational delirum, in which, according to the classic descriptions, the patient believes he performs his usual work and is engaged in imaginary pursuits, fortifies himself with a drink, etc., seems nowadays to have be superceded by a far more undemonstrative and appreciably less picturesque condition. The patient has visual hallucinations of the same type as in the premonitory phase, but the hallucinations of the full-blown delirium do not occur only when the patient is about to fall asleep. The patient believes in the reality of his hallucinations; he sees small animals in threateningly large numbers or persons scolding him. His response to these phenomena is often that he jumps out of bed and runs to the window to throw himself out, or, in rare cases, that he attacks his surroundings.

Abnormal skin sensations, such as insect bites, formication or a feeling as if the bed were filled with broken glass, are often experienced. New hallucinations may readily be excited by suggestion. On the whole, these patients are highly suggestible; they may, for example, be induced to read from a sheet of blank paper.

The delirious patient is usually docile, with some sort of sardonic humour, and rarely resorts to actual violence.

The duration of untreated delirium tremens is usually three to five days (2), sometimes a week or more. During this time the condition is fluctuating. In the evening, aggravation with intensified anxiety often occurs. The insomnia is very stubborn. The patient suffers from sweats and congestion, and fever up to 38—39° C may develop. Pronounced electrolyte imbalance and, most frequently, albuminuria are present. Untreated delirium tremens commonly runs a fatal course. The most frequent cause of death is pneumonia with circulatory failure. The patients who recover pass through a critical phase, fall into a profound sleep and are fairly lucid on awakening, retaining a faint recollection of the delirious hal-

lucinations. In some cases, chronic alcoholic psychoses, *e. g.* Korsakow's psychosis, may result.

TREATMENT

In Denmark, the treatment of delirium tremens with veronal has given excellent results. Shortly after von Mering and Fischer had released this drug for therapeutic experiments (1903), Jacobson, of Frederiksberg Hospital, gave it a trial in delirium tremens (4, 5). After some experiments, he began to administer veronal on the lines which are still followed. In our hospital, we give 0.75 to 1.0 g of veronal up to three times at hourly intervals. It is extremely important that the drug is given as early as possible after admission. At least we do not, for academic reasons, await the development of classic delirium, the presence of which secures the diagnosis, but at the same time materially reduces the possibility of complete recovery. The physical condition of the patient should be kept under close observation. Injections of vitamin-B concentrates and, as a prophylactic measure, also of penicillin are given. Fluid therapy is rarely necessary. On this medication, the badly needed sleep is usually induced, lasting from six to twelwe hours. On awakening, the patient is most frequently lucid and quiet, but often still trembling. Another dose of veronal (0.25—0.5 g) is then given. During the following days, a similar dose is administered in the evening. In fully developed delirium, the same initial dose (0.75—1.0 g up to three times at hourly intervals) is employed. With this treatment, the condition runs a smoother course, but its duration is scarcely materially shortened.

It is important to emphasize that the patients should be treated in hospital, with as few restrictions as possible. The light should be kept on at night.

The results obtained by Jacobson were published by Friis Møller. From the discussion which followed, it appears that in some hospitals it was impossible to reproduce these results. There is reason to believe that the principal cause of this was the one stated by Friis Møller (7), *viz.*, that the patients at Frederiksberg Hospital were treated very early, *i. e.*, already while the premonitory symptoms were present.

The superiority of this treatment was emphasized by Ranson & Scott (8) in 1911. They pointed out that veronal was the most efficacious drug available, and, especially, that this drug was capable of preventing the development of florid delirium tremens, but they also believed to have shown that it was able to reduce the mortality from fully developed delirium.

Since then, two generations of psychiatrists in Denmark have found that veronal is the drug of choice in the treatment of delirium tremens, whereas this method seems to have fallen into oblivion in other countries. In several comprehensive studies, the drug is not even mentioned, which seems sufficient to justify the appearance of this paper.

In this connection it is tempting to cite a statement of an "experienced" chronic alcoholic, who had had several attacks of delirium tremens and had been treated in several parts of the world before he was admitted to our hospital with a new attack. When he had recovered, he declared that he was very satisfied with "this new veronal tratment, because it was much better than the old-fashioned reserpine methods".

SUMMARY

After the introduction of appropriate social measures against the abuse of alcohol, especially in the form of heavy taxation, delirium tremens has become a rare disease in Denmark. The cases which do occur usually present no special therapeutic problems.

The explanation of the therapeutic success is believed to be:

1. Early recognition of delirium tremens or of its potential development.
2. Early intensive veronal treatment both in actual cases and, prophylactically, to patients in the "danger zone", accompanied by adequate treatment of any coexisting somatic disease.

The clinical feature of the early stages of the disease are considered in some detail. It is emphasized that fully developed delirium tremens should not, for academic reasons, be awaited, since this materially reduces the chances of complete recovery.

The treatment consists in administration of veronal, 0.75 to 1.0 g up to three times at hourly intervals, which usually induces sleep. During the next few days, 0.25—0.5 g of veronal is given two or three times.

By this treatment, incipient delirium is checked, and the fully developed condition runs a smoother course, but the duration of the disease is hardly materially shortened.

Two generations of Danish psychiatrists have found that veronal, administered as outlined by Jacobson, is the drug of choice in the treatment of delirium tremens.

This investigation has been supported by a grant from Ford Foundation.

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ROUTINE DIAGNOSIS OF ENTEROVIRUSES USING TISSUE CULTURE TECHNIQUES

EXPERIENCES IN DENMARK DURING 1956—1958

By ANNELISE GODTFREDSEN and HERDIS von MAGNUS

Since 1956 examination of stool specimens for polio, echo, and Coxsackie viruses has been carried out in this institute on a par with other diagnostic tests.

During 1956—1958 only few cases of paralytic polio occurred in Denmark and the majority of stool specimens examined during this period derived from patients admitted to the hospitals with a diagnosis of meningitis or aparalytic poliomyelitis.

The present paper reports the results obtained in this period by routine tests for polio- and echoviruses using cultures of trypsinized monkey kidney cells.

MATERIAL AND METHODS

Fecal specimens were sent to our laboratory in special containers by ordinary mail and were usually received on the following day.

Tissue cultures. Roller tube cultures of trypsinized monkey kidney cells were employed. For preparation of the tubes the method described by Younger (1) was used with some modifications (2).

The nutrient medium during outgrowth of cells was 0.5 per cent lactalbuminhydrolyzate in Hank's solution with either 2 per cent horse serum or 1 per cent calf serum. Prior to inoculation — usually on day 4 — this medium was replaced by 1.8 ml of Medium 199. All nutrient media contained either penicillin (100 μ/ml) plus streptomycin (0,1 mg/ml), or nebacetin 0.1 mg/ml.

Preparation of fecal suspensions. As a rule the stools were prepared for inoculation immediately after being received. Occasionally, however, the specimens were kept for 24—48 hours at + 4°C before treatment was initiated.

A 10 per cent stool suspension in saline was prepared from approximately 0.7 g of fecal material, and the mixture was placed overnight at — 20°C. Next morning the fluid was thawed and centrifuged at 1000 r. p. m. for 15 minutes. The supernatant was removed and spun at 3000 r. p. m. for 30 minutes. The supernatant fluid from the last centrifugation was used for inoculation of roller tubes.

If the stools were found to contain organisms resistant to the antibiotics employed in the tissue culture system, the stool suspensions were treated with ether in the following way: A mixture of one part of ether and two parts of stool suspension was placed overnight at + 4°C. The ether was subsequently removed by evaporation before inoculation of the suspension into tissue culture tubes.

Technique for virus isolation in tissue culture:

To each of 3—5 roller tubes, 0.2 ml of stool suspension was added. In order to reveal possible cross contamination with virus in the experiments, the following precaution was taken: For each set of tubes inoculated with stool material, three tubes were left uninoculated. The next 3—5 tubes were inoculated with another stool suspension, then followed again 3 control tubes and so on. All tubes were placed in roller drums at 37°C. After three hours the fluid was removed from both the inoculated tubes and the control tubes and replaced by 1.8 ml of bovine amniotic fluid. After five days the nutrient medium was again renewed.

The cultures were examined microscopically for cytopathic changes on day 5, 9 and 14 after inoculation and culture fluid from tubes showing cell lesions was harvested for further study.

During the first 10 months of the study period blind passages were carried out from tubes negative on day 5. Since, however, only one out of a total of 477 such blind passages resulted in isolation of a virus strain from tubes remaining microscopically normal through the 14-day observation period, this blind-passage procedure was discontinued, and subsequently all cultures remaining normal for two weeks were regarded as negative.

Whenever it was of particular interest to establish whether a small amount of virus might be present in a stool from which no virus had been isolated on the first attempt, the test was repeated using 10 tubes per sample.

Typing of virus strains isolated:

Hyperimmune sera: The preparation of polio antisera and echo antisera has been described in an earlier paper (3). Coxsackie antisera had been prepared by hyperimmunization of hamsters and mice.

Neutralization tests with polio antisera: During 1956 and the beginning of 1957 the cytopathogenic agents isolated were tested only in neutralization tests with polio antisera. As a routine

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procedure tissue culture fluid was diluted 1:100 and mixed with equal amounts of polio antisera against each of the three types of poliovirus as well as with a pool containing all three sera, each serum being used in a final dilution of 1:200. The titres of these sera were 2048 or more against the homologous virus.

After incubation at room temperature for one hour two roller tubes were inoculated with each mixture, an inoculum of 0.2 ml. being used for each tube. Virus controls and serum controls were included in each test. As a rule the tubes were read three and seven days after inoculation. If the result of a neutralization test was not unequivocal because too many virus doses had been employed, the neutralization test was repeated with virus material diluted 1:1000 or more and a virus titration was included to determine the number of virus doses actually used.

Neutralization tests with echo antisera: The majority of virus strains isolated from stools during 1956—1957 was not neutralized by polio antisera and since it was found that many virus strains recovered from spinal fluids during this period belonged to the echo 9 group of viruses (3), all strains isolated from stool specimens which were not neutralized by polio antisera were later tested in neutralization tests against echo antiserum type 9. From June 1957 all strains isolated were screened in neutralization tests using echo antiserum type 9 as well as the three polio antisera in the test.

Virus strains which were not neutralized by any of these antisera were in some instances examined in neutralization tests with echo hyperimmune sera types 1 through 14. All echo antisera were used in final dilutions corresponding to approximately 20 units of antibody and the result of the neutralization test was based on the seventh day reading of the tubes inoculated with approximately 100 TCD_{50} (at least 50 TCD_{50}). Since no predetermination of the virus titer had been made, each test included several virus dilutions and a parallel virus titration was included in each test.

Inoculation of suckling mice with tissue culture virus: Tissue culture passage material of virus strains isolated during 1956—1957 and not belonging to the polio group of viruses were with few exceptions tested by inoculation into newborn mice. In 1958 only strains which were not typable as poliovirus or echovirus type 9 were tested by inoculation into newborn mice.

Neutralization tests with Coxsackie antisera: Virus strains which were found to induce paralysis in newborn mice and which were not neutralized in tissue culture tests by echo antiserum type 9 were tested in neutralization tests in newborn mice. Equal amounts of undiluted unknown virus and each of the Coxsackie antisera types A-9, B-1, B-2, B-3, B-4 and B-5 diluted 1:4 were mixed and incubated for 1 hour at room temperature. Each mixture was inoculated into one litter of newborn mice. The mice were observed daily for seven days.

RESULTS

Virus isolations from patients with paralytic poliomyelitis

During 1956—1958 a total of 120 cases of paralytic polio, *i. e.* 39 in 1956, 15 in 1957, and 66 in 1958, was reported in Denmark.

Information kindly supplied by the hospitals concerning these patients revealed, however, that the preliminary diagnosis of paralytic polio had in some cases not been supported by the subsequent clinical course. Thus for 12 patients the initial diagnosis could not be maintained, and the revised figures are thus 34, 9 and 65 paralytic polio cases for 1956, 1957 and 1958, respectively. The results of virus isolation studies are listed in Table 1.

Table 1.

Virus isolations from paralytic polio patients in Denmark during 1956—1958.

	Number of patients	Polio virus type I	II	III	Virus not isolated	No specimens received
1956	34	24	1	0	9	
1957	9	5	1	0	3	
1958	65	15	0	32	16	2
Total	108	44	2	32	28	2

In 1956 24 type I strains and one type II poliovirus strain were isolated from the 34 paralytic polio patients.

From the nine paralytic patients in 1957 six polio strains were isolated (5 type I and one type II).

From two of the 65 patients reported as paralytic polio in 1958 no stool specimens were received. Of the remaining 63 patients 47 were found to excrete poliovirus (32 type III and 15 type I).

From 28 out of 106 patients with a clinical diagnosis of paralytic polio no virus could be recovered from the stools.

Virus isolations from patients with meningitis or aparalytic polio.

Stool specimens from 1344 patients admitted to the hospitals in 1956—1958 with a diagnosis of meningitis or aparalytic polio were examined by tissue culture techniques. The results obtained are shown in Table 2.

Cytopathogenic agents were recovered from a total of 264 patients (19 per cent). Poliovirus type I was isolated from 17 patients, poliovirus type II from one patient, poliovirus type III from nine patients, echovirus type 9 from 152 patients, and Coxsackie virus from 27 patients.

Table 2.

Monthly distribution of enteric viruses recovered from stool specimens from patients with an initial diagnosis of aseptic meningitis or aparalytic poliomyelitis.

Year	Month	Number of patients examined	Number positive	Poliovirus type I	Poliovirus type II	Poliovirus type III	Echovirus type 9	Echovirus other types	Coxsackie virus A-9	Coxsackie virus B-1	Coxsackie virus B-2	Coxsackie virus B-4	APC virus	Strains unidentified to date
1956	Jan.	4	1				1							
	Febr.	7	0											
	March	12	0											
	April	6	1										1	
	May	10	0											
	June	11	0											
	July	47	2	1									1	
	Aug.	62	12	1	1		6	1						3
	Sept.	151	31	7			19		1		2			2
	Oct.	168	55	1			44				2			8
	Nov.	106	20	2			15				1			2
	Dec.	67	14	1			11							2
	Total	651	136	13	1	0	96	1	1	0	5	0	2	17
1957	Jan.	54	4				3							1
	Febr.	27	2				2							
	March	25	0											
	April	18	1											1
	May	17	2				1							1
	June	18	3				2							1
	July	31	8				4		1					3
	Aug.	62	14	1			5	1	1				1	5
	Sept.	68	4				4							
	Oct.	43	3				2				1			
	Nov.	18	4				3							1
	Dec.	10	4				2							2
	Total	391	49	1	0	0	28	1	2	0	1	0	1	15
1958	Jan.	13	2				2							
	Febr.	13	2				1							1
	March	12	0											
	April	10	2				1						1	
	May	11	0											
	June	10	1										1	
	July	24	4			1				3				
	Aug.	54	12				7			2				3
	Sept.	54	22	1		4	1			9		1		6
	Oct.	37	15	1		2	6			3				3
	Nov.	41	16	1		2	9							4
	Dec.	23	3				1							2
	Total	302	79	3	0	9	28	0	0	17	0	1	2	19
Total		1344	264	17	1	9	152	2	3	17	6	1	5	51

One strain of echovirus type 6 was isolated in 1956 and in 1957 one patient was found to excrete echovirus type 5.

Some of the agents isolated induced cytologic alterations of a type characteristic for the adenovirus group (4). Two of these agents were identified in neutralization tests as adenovirus type 3, one strain as adenovirus type 5, and two strains as adenovirus type 7.*)

From 51 patients 61 virus strains listed as "unidentified virus strains" were isolated. This group represents agents causing cytopathic lesions in monkey kidney tissue culture that are neither neutralized by a pool of polio antisera nor by echo antiserum type 9. Fourteen of these 61 strains were found to be pathogenic for newborn mice after passage in tissue culture. So far, only five of these strains have been tested in neutralization tests in newborn mice with Coxsackie antisera types A-9 and B-1 to B-5. None of them was neutralized by any of these sera.

Of the remaining 47 strains which were found not to be pathogenic for newborn mice, 16 have so far been examined in neutralization tests with echo antisera types 1 through 14; the agents were not neutralized by any of these sera.

Mouse pathogenicity of echo 9 strains isolated during 1956—1957.

As will be seen from Table 2 echovirus type 9 was during 1956—1957 recovered from stools

*) We are indebted to Dr. *Knud Birkum Petersen* of this institute for the typing of these strains.

from a total of 124 patients. Since in many instances more than one stool specimen was received from each patient, this represents a total of 145 echo 9 isolations during 1956—57. Of these echo 9 strains 137 were tested by inoculation of newborn mice, the second tissue culture passage usually being employed. The results of these tests are shown in Table 3. It will be seen that tissue culture material from 137 of these echo 9 strains in 83 instances (60 per cent) produced paralysis in newborn mice.

Table 3.

Mouse pathogenicity of echo 9 strains isolated in tissue culture during 1956 and 1957.

Number of echo 9 strains isolated in TC	Result of inoculation of newborn mice with TC-fluid		Result of inoculation of corresponding 10 % stool suspension into newborn mice positive	negative	not done
	pos.	83	3	41	39
145	neg.	54	0	30	24
	not done	8	0	4	4
			3	75	67

It was found that stool specimens from three of the patients excreting echovirus type 9 induced paralysis in newborn mice also by primary inoculation, without previous tissue culture passage. In order to rule out the possibility of the presence of a mixture of echo 9 virus plus another virus strain in the stools the following experiments were carried out: Carcasses from mice paralyzed after inoculation with stool suspension were harvested, titrated in newborn mice and tested by neutralization test in newborn mice against echo antiserum type 9 diluted 1:10 and 1:100. Approximately 100 MID_{50} were employed in the experiment. Two litters of newborn mice were inoculated with a mixture of virus and normal monkey serum diluted 1:10 and 1:100 to serve as controls. None of the mice inoculated with a mixture of virus and echo antiserum type 9 showed any symptoms seven days after inoculation whereas all animals inoculated with a mixture of virus and serum from normal monkeys were paralysed five to six days after inoculation.

It could thus be demonstrated that the paralysis of the mice was actually caused by the echo 9 virus present in the stools.*)

The content of echovirus in the stools is usually rather low (8) and the titer in the stools during the first week of illness has in our laboratory never been found to exceed $10^{-2.5}$. The stool suspension from one of the patients has been titrated simultaneously in newborn mice and in tissue culture, and the titers were found to be $10^{-1.1}$ and $10^{-1.3}$, respectively. The primary pathogenicity for newborn mice thus cannot be explained by an unusually large amount of echo 9 virus in the stools.

Comparison of a current echo 9 strain and the prototype echo 9 strain.

As already mentioned, a new strain was classified as echovirus type 9 if the agent was completely neutralized on the seventh day in a neutralization test where approximately 100 TCD_{50} of virus and 20 units of antibody were used.

Since several dilutions of a virus strain were included in each neutralization test it was very often noted that while 20 units of antibody were able to inhibit the cytopathogenic effect of as much as 5000 virus doses of the prototype echo 9 virus, the protective effect against some of the freshly isolated strains was less pronounced, *i. e.*, when approximately 1000 TCD_{50} of freshly isolated strains were employed, neutralization was clearly evident by the third day reading but not on the seventh day reading.

The most likely explanation for these observations seemed to be that the Danish echo 9 strains and the prototype echo 9 strain were antigenically slightly different.

One of the current echo 9 strains (strain 4682 S) and the prototype echo 9 strain were therefore studied in cross neutralization tests using both homologous and heterologous sera. Strain 4682 S had been isolated from a spinal fluid, and it was non-pathogenic for newborn mice also after passage in tissue culture.

The hyperimmune sera were prepared as follows: For each strain one rabbit was inoculated three times intravenously with weekly intervals, one ml of undiluted tissue culture virus being inoculated each time. The virus titer of these suspensions was $10^{-6.6}$ for the prototype echo 9 strain and $10^{-7.3}$ for strain 4682 S. The animals were bled one week after the last inoculation.

Two-fold dilutions of each serum were tested against approximately 50 TCD_{50} of each virus strain. Each serum-virus mixture was inoculated into 4 roller tubes, an inoculum of 0.2 ml being used for each tube. Parallel virus titrations were carried out and the serum and virus titres were read after seven days. Several titrations on each serum have been carried out with corresponding results. A representative example is shown in Table 4. As will be seen, a high neutralizing antibody titer could be demonstrated in both sera

Table 4.

Cross-neutralization antibody end points with hyperimmune sera of strain 4682 S and the prototype echo 9 strain (Hill).

Strain of virus	TCD_{50}	Reciprocal of final serum dilution 4682 S	Hill
4682 S	60	22390	152
Hill	40	360	2048

*) The serum used for identification was kindly supplied by The National Foundation for Infantile Paralysis through the coutesy of Dr. *H. A. Wenner.*

against the homologous virus whereas the titer against the heterologous virus strain was significantly lower.

DISCUSSION

This paper presents the results of diagnostic tissue culture tests for enteric viruses on fecal specimens during a three-year period. The material studied derived from patients with symptoms indicating an infection of the central nervous system; all specimens were sent from the hospitals on a routine basis.

Tissue cultures of trypsinized monkey kidney cells were used throughout the study. Such cultures are at present considered to represent one of the most sensitive systems for the isolation of enteric viruses. The cultures are, however, rather vulnerable to the toxic effect exhibited by many stool suspensions. Also, non-specific degeneration of the cells is often seen when kidney cells are maintained with synthetic medium 199 during the period of observation, which is two weeks, even when the medium is changed.

In order to overcome these difficulties, two precautions have been taken: Firstly, three hours after inoculation of the tubes with the stool suspension, the fluid is removed and replaced with fresh medium. Experiments carried out in our laboratory (unpublished data) have indicated that a three-hour contact period is sufficient to allow the demonstration of even minimal amounts of poliovirus present in the stools. This observation is in accordance with results obtained by others (5).

Secondly, bovine amniotic fluid has been used as replacement for synthetic medium 199 from the time of fluid change three hours after seeding of the tubes and during the rest of the observation period. This fluid is, as pointed out by Enders (6), an excellent nutrient medium for tissue culture work, and the monkey kidney monolayer cells usually remained in healthy condition with this maintenance medium.

During the 3-year period 1956—1958 stool specimens from a total of 106 patients with a clinical diagnosis of paralytic polio were examined. In 78 instances (74 per cent) the clinical diagnosis was supported by isolation of poliovirus from the patients. However, examination of stool specimens from the remaining 28 patients gave negative results.

Five of these virus-negative patients had facial paralysis as the only paralytic sign. In our experience poliovirus can only seldom be demonstrated in the stools from such patients. During a four-year period, 1955—1958, stool specimens were received from a total of 18 patients suffering from isolated paralysis of the facial nerve. In four instances the fecal specimens were unfortunately collected too late in the disease to allow any conclusions to be drawn from the negative results obtained. However, from the remaining 14 of the patients with facial paralysis the first stool sample was collected during the first or second week of illness, and only one of these patients was found to excrete poliovirus (type I). These findings are similar to those published by Zacek et al. (7); these workers were thus able to recover poliovirus from only 13 out of 70 patients suffering from isolated paralysis of the facial nerve.

In another respect our findings differ from those of the Czechoslovakian investigators. Thus, Zacek et al. (7) have in some instances been able to demonstrate the presence of poliovirus in stools by blind passage from inoculated tubes showing no cytopathic cell lesions. In our hands poliovirus has never been demonstrated by this method. As mentioned previously, only in one out of more than 400 instances, blind passage carried out five days after inoculation from tubes remaining normal through the observation period of 14 days was found positive. The virus strain isolated by this method was not neutralized by polio antisera and it has so far not been identified.

As regards the negative results obtained for the remaining 23 patients with other types of paralysis, some may probably be explained by the fact that the stool samples were collected later than 30 days after the onset of illness. Also, considerable individual variations were observed as regards the duration of virus excretion as well as in the amount of poliovirus excreted in the stools, even during the first week of illness. Titrations of the virus content in early stool samples from 12 patients thus gave values ranging from $10^{-0.8}$ to $10^{-4.5}$ TCD_{50}. In some patients virus could not be demonstrated beyond the first week, while four patients were found to excrete virus for more than 75 days. The study material is very non-homogeneous, however, and thus not suited for any conclusions concerning factors influencing the excretion of virus in the stools.

Of a total of 1344 patients with a tentative diagnosis of aseptic meningitis, virus was isolated from 264. This figure is rather low (19 per cent). It should be emphasized, however, that this study is based on "routine samples" and that further information concerning the patients with a tentative diagnosis of aseptic meningitis was usually not obtained. As already mentioned, shifts in diagnosis occurred in 12 out of 120 cases where the patients were admitted to the hospitals under a diagnosis of paralytic polio. In the meningitis group of patients shifts in diagnosis have probably occurred even more often, and the isolation-frequency of 19 per cent must therefore be considered a minimum figure for enteric virus infections in the patients with aseptic meningitis. And — as was also the case for the paralytic patients — in many instances the stool specimens were collected rather late in the disease.

Stool specimens from "normal controls" were not examined during this period, and only limited conclusions can therefore be drawn concerning the etiologic relationship between the virus strains isolated and the patients' illness.

The majority of agents isolated from the "aseptic meningitis" cases could be classified as echovirus type 9, which accounted for 152 (58 per cent) of the virus isolations. However, although this virus was dominating, it was recovered from only 11 per cent of the aseptic meningitis cases studied during the three-year period. That this low figure is not due to inappropriate virus isolation technique is indicated by the fact that during a small localized outbreak of aseptic meningitis in Jylland in 1956, echovirus type 9 was recovered from 10 out of 12 patients examined.

By cross-neutralization tests between a Danish echo 9 strain and the prototype echo 9 strain a difference in antigenic structure between the two strains was demonstrated. Isolation of echo 9 strains antigenically different from the prototype "Hill" strain has been reported by Sabin (8) and by Swedish workers (9). In contrast to the echo 9 strains isolated during 1956 in Sweden (9), the Danish echo 9 strain did not seem to be antigenically broader than the prototype echo 9 strain. Thus hyperimmune serum prepared with the Danish strain had a low neutralizing antibody titer against the prototype echo 9 strain, whereas a high antibody titer against the homologous virus could be demonstrated.

Approximately 60 per cent of the Danish echo 9 strains were found to be pathogenic for newborn mice after passage in tissue culture. Isolation of echo 9 strains pathogenic for newborn mice after tissue culture passage has been reported by several authors (for references: Berger and Melnick (10)). It seems, however, of interest that stool suspensions from 3 of the Danish patients excreting echo 9 virus produced paralysis in newborn mice without previous passage in tissue culture. The patients were all children infected in 1956 during the small endemic in Jylland mentioned previously in this paper.

This seems to be the first observation reported on the occurrence of "primary" mouse-pathogenicity of echo 9 virus strains.

Of the remaining 112 "virus-positive" patients only 27 were found to excrete poliovirus. Since the virus group thus accounted for the infection of only two per cent of the 1344 patients with a tentative clinical diagnosis of aseptic meningitis or aparalytic polio, it seems justified to conclude that during 1956—58 poliovirus played only a very minor role in the etiology of aseptic meningitis in Denmark.

Various types of Coxsackievirus, adenovirus and echovirus were isolated from 34 patients (three per cent of the group) while 51 patients were found to excrete cytopathogenic agents which have not been identified by the studies carried out to date.

SUMMARY

The tissue culture methods used during 1956—1958 in our laboratory for routine diagnosis of enteric viruses in the stools have been described.

All specimens examined were derived from patients admitted to the hospitals under a diagnosis of paralytic or aparalytic polio or aseptic meningitis.

From 106 patients with a clinical diagnosis of paralytic polio 78 polio strains were recovered (44 type I, 2 type II and 32 type III).

Out of 1344 patients with an initial diagnosis of aparalytic polio or aseptic meningitis, only 264 (19 per cent) were found to excrete enteric viruses. More than half of the strains isolated, 152 in all, belonged to the echo 9 group of viruses. Only 27 patients were found to be infected with poliovirus. A variety of types of Coxsackie, adeno- and echoviruses accounted for 34 strains, while 51 agents have so far not been identified.

Sixty per cent of the isolated echo 9 strains were found to be pathogenic for newborn mice after passage in tissue culture. Three echo 9 strains were found to be pathogenic for newborn mice without previous passage in tissue culture.

A difference in antigenic structure between the prototype echo 9 strain and a Danish echo 9 strain has been demonstrated.

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PROTAMINE-ZINC-INSULIN IN CRYSTALLINE SUSPENSION

By CH. KRAYENBÜHL and J. E. POULSEN

Advances in the chemistry of long-acting insulins make it possible to produce insulin preparations with great variations in the timing of the effect on the blood sugar.

In many cases it is necessary to have insulin preparations with the most speedy effect on the blood sugar, such as the unmodified insulin in solution. This preparation is indispensable during coma and in some cases of intercurrent diseases in diabetics, who may usually be satisfactorily regulated with one daily injection of a preparation with an intermediary or slow effect. Therefore unmodified insulin must always be available for the treatment of diabetes.

The aim of the insulin laboratories has been to elaborate preparations suitable for the greatest percentage of diabetics, and it has been found that preparations with an intermediarily prolonged effect, such as Protamine-Insulin Retard NPH (Insulinum isophanum) or Insulin Lente, are suitable for a very great number of diabetics who take one injection daily.

Sometimes, however, especially in juvenile cases, it is not possible to obtain a good control of the diabetic state with one injection daily of a single preparation. In such cases it is an advantage to combine the quick effect of the unmodified insulin with the more protracted effect of insulin with intermediary or slow effect, and it has been shown that the crystalline suspension of Protamine-Insulin Retard NPH can be mixed in the syringe before injection with unmodified insulin without noticeable change in the characteristic timing effect of each of the two components of the mixture.

After the development of Protamine-Insulin by Hagedorn et al. (7) the Protamine-Zinc-Insulin (PZI) was introduced by Fisher & Scott (6) and has been used since 1936; it has a later starting and more protracted effect on the blood sugar than Protamine-Insulin Retard NPH. The original PZI, the production of which is described in the American and British pharmacopoeias, is a suspension of a primarily amorphous precipitate of insulin with protamine and zinc, and generally some unpredictable degree of protamine insulin crystallisation takes place in the precipitate during storage. This preparation may therefore change during storage, the suspended particles being inhomogeneous.

The PZI has very often been used in mixture with regular insulin, and such mixtures are discussed in the leading monographs (1, 2, 3, 4, 5, 8) on the treatment of diabetes mellitus. The drawback of such a mixing procedure has been that the greater part of the added regular insulin has been precipitated as PZI and thus acquired a timing effect similar to PZI. On account of the uncertain degree of crystallization of the PZI, the binding of added regular insulin varies rather much.

In the Nordisk Insulinlaboratorium a new method for production of a slow-acting PZI-modification has been developed, resulting in a uniform crystalline suspension of insulin, protamine, and zinc. The preparation is buffered with phosphate as the usual PZI, and the precipitate contains all the insulin, protamine, and zinc present. The amounts of all components are within the limits stated in the said pharmacopoeias regarding the total composition.

The reason why the new PZI is better suited for mixing with unmodified insulin is that only a minor part of the latter is bound during the mixing. This characteristic is exceedingly good for all proportions when unmodified insulin in neutral solution (*i.e.* pH: 7.3, similar to PZI) is used for the mixed preparation. The crystalline PZI, however, is sufficiently buffered to allow mixtures of up to at least three parts of the acid unmodified insulin to one part of PZI. Higher proportions of unmodified insulin to PZI may change the pH to the isoelectric zone of insulin.

In order to avoid any risk of enzymatic decomposition of the insulin it has been generally claimed that unmodified insulin must be prepared as an acid solution, but this should not be necessary when high purification of the insulin is developed.

The following figures demonstrate the amount of unbound insulin in different mixing proportions between PZI and unmodified insulin in solution. The abscissa gives the fraction of PZI to unmodified insulin for a total amount of 40 units per ml. The ordinate shows the amount of unbound insulin in units per ml.

METHOD

After mixing the two preparations in certain proportions, the crystalline PZI-precipitate is removed by centrifugation, and the insulin content in the centrifugate is determined. A rather simple way of measuring the quantity of insulin is to fix the amount of protamine necessary for isophane precipitation of the insulin present in certain volumes of the centrifugate. The protamine is added as a solution with a known content of protamine-sulphate. The principle of the isophane-determination has been described earlier (9). Under predetermined conditions, such as temperature, concentration of buffer and pH, the amount of protamine required for isophane precipitation of insulin is directly proportional to the amount of insulin.

From the Nordisk Insulinlaboratorium and the Steno Memorial Hospital, Gentofte — Denmark.

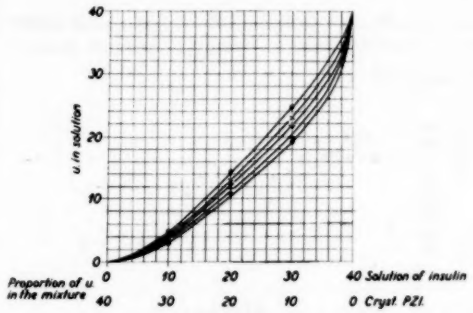

Fig. 1.

° *5 min. after mixing*
x *15 min. after mixing*
▽ *30 min. after mixing*
+ *2 hours after mixing*
• *6 hours after mixing*

Fig. 1 shows the amount of free insulin in the mixtures when the suspension of crystalline PZI and the solution of unmodified insulin have the same pH: 7.3, so that no change in pH occurs during mixing. It appears from the curves that only a minor part of the insulin added has been bound. The binding of free insulin by mixing does not occur instantaneously. The rate of binding, however, decreases considerably within five minutes.

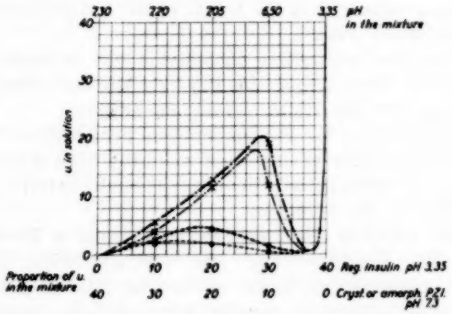

Fig. 2.

The two lower curves, obtained 5 and 30 minutes respectively after mixing, demonstrate the low amount of free regular insulin in mixture with amorphous PZI.
The upper curves obtained with the same intervals demonstrate the great amount of free regular insulin when mixed with crystalline PZI.

Fig. 2 shows the amount of unbound insulin when crystalline and amorphous PZI is mixed with unmodified insulin in acid solution, i.e. regular insulin.

The change in pH is correlated to the proportion of mixture, and when it amounts to three parts of unmodified insulin (pH: 3.3) to one part of crystalline PZI (pH: 7.3) the isoelectric precipitation zone of the insulin will be reached (the lower right part of curves in fig. 2).

The use of the acid solution of the unmodified insulin in the mixtures reduces unbound insulin a little, as the precipitating power of the protamine present will be greater when the pH approaches the isoelectric zone of the insulin. The continuous transition to complete isoelectric precipitation of the unmodified insulin under the particular mixing proportions at about pH: 5.5 does not, however, prevent the quick acting of this insulin.

CLINICAL EFFECT

The effect of the crystalline PZI on the blood sugar is identical with that of the classical PZI. The clinical advantage of the crystalline PZI is that because of its stability as suspension it can be mixed with both unmodified insulin and Protamine-Insulin Retard NPH, and it is at least theoretically possible to adapt an individual mixture of these three preparations to comply with the changing insulin requirements of diabetics through a 24-hour period with one single injection of the mixture.

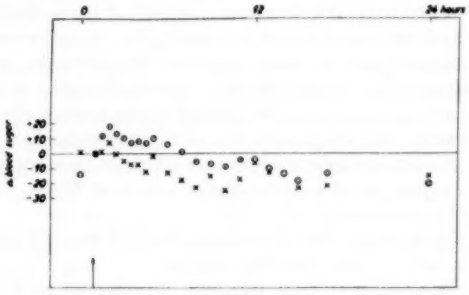

Fig. 3.

Male patient, 19 years old. Diabetes mellitus since his 9th year.
° ° ° *Protamine-Zinc-Insulin LEO (crystalline) 24 u.*
x x x *Protamine-Zinc-Insulin LEO (primarily amorphous) 24 u.*

The following figures show the effect of the new crystalline Protamine-Zinc-Insulin and the timing effect of mixtures of the new preparation with Insulin NPH and regular insulin.

On the days of the examinations the patients were confined to bed. They got 300—400 grams

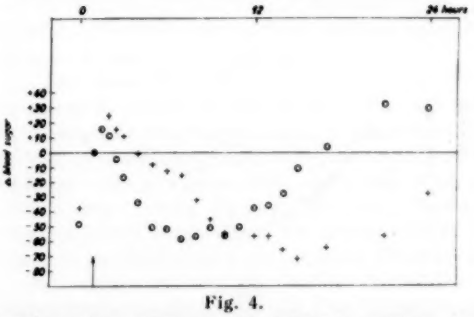

Fig. 4.

Female patient, 17 years old. Diabetes mellitus since her 9th year.
° ° ° *Protamine-Zinc-Insulin LEO, 12 u. + Insulin LEO, 12 u., given mixed in the syringe.*
+ + + *Protamine-Zinc-Insulin MCO, 12 u. + Insulin LEO, 12 u., given mixed in the syringe.*

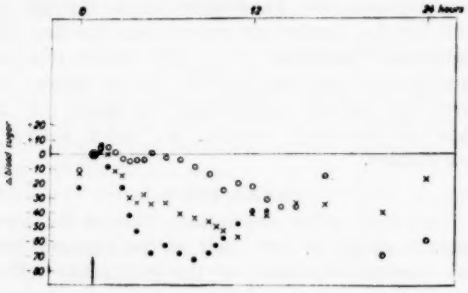

Fig. 5.

Male patient, 19 years old. Diabetes mellitus since his 9th year (same patient as Fig. 3).

° ° ° *Protamine-Zinc-Insulin LEO (crystalline) 24 u.*

x x x *Insulin Retard NPH LEO (Insulinum isophanum) 24 u.*

• • • *Insulin LEO, 16 u. This curve from a female patient. 17 years old. Diabetes mellitus since her 10th year.*

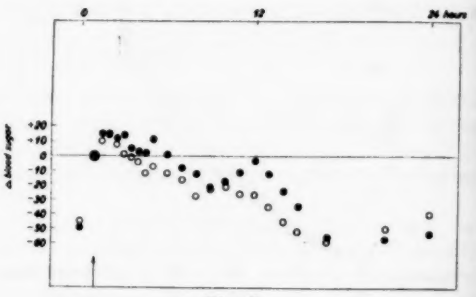

Fig. 7.

Male patient, 22 years old. Diabetes mellitus for 4 years (same patient as Fig. 6).

° ° ° *Protamine-Zinc-Insulin LEO (crystalline) 8 u. + Insulin Retard NPH LEO (Insulinum isophanum) 8 u., given simultaneously in 2 injections.*

• • • *The same dose as above, given mixed in the syringe.*

of vegetables and 10 grams of butter three times daily (between hours 1—2, 5—6, 10—11 after the beginning of the experiment). The insulin injections were given at *Hour one* (indicated with an arrow). Intervals of at least three normal days between the examinations were maintained.

The blood-sugar curves are given as percentage variations of the blood-sugar value at *Hour one* (Δ blood sugar).

Fig. 3 shows the identical effect of the old and the new Protamine-Zinc-Insulin.

Fig. 4 shows that mixture of crystalline PZI LEO and regular insulin has a quicker and less protracted effect than the corresponding mixture of pure amorphous PZI and regular insulin.

Fig. 5 shows the typical timing effect on the blood sugar of the three different Insulin LEO preparations.

Fig. 6 shows the identical effect of separate injections of unmodified insulin and crystalline PZI, and of a mixture of the said preparations.

Fig. 7 shows the identical effect of separate injections of Insulin Retard NPH LEO and of crystalline PZI, and of a mixture of the said preparations.

Fig. 6.

Male patient, 22 years old. Diabetes mellitus for 4 years.

° ° ° *Protamine-Zinc-Insulin LEO (crystalline) 8 u. + Insulin LEO, 8 u., given simultaneously in 2 injections.*

• • • *The same dose as above, given mixed in the syringe.*

SUMMARY

A Protamine-Zinc-Insulin preparation as a primarily crystalline suspension has been described.

The preparation buffered with phosphate buffer has the content of protamine, zinc, and other components within the limits of the old primarily amorphous PZI.

The new crystalline suspension has crystals of uniform shape, and the crystals do not change during storage under usual conditions.

Contrary to the old amorphous preparation the new crystalline preparation will only bind a small part of unmodified insulin, if such insulin is added to the solution.

The clinical effect on the blood sugar is identical for the new PZI and the old PZI. The new preparation, however, can be mixed in the syringe both with regular (unmodified) insulin and/or Protamine-Insulin NPH without any noticeable change in the characteristic timing of the effect of the components of such mixtures on the blood sugar.

We wish to thank Mrs. *M. Schou*, Dr. pharm., for her valuable technical assistance in the chemical work.

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